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(FILE 'HOME' ENTERED AT 11:22:07 ON 12 FEB 2002)

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
11:22:15 ON 12 FEB 2002

L1 13924 S AUXOTROPHIC OR AUXOTROPH
L2 301 S L1 AND BACTERIOPHAGE
L3 124 S L2 AND (ACID? OR FERMENT? OR CULTURE)
L4 1062753 S L3 AND BACTERIA OR BACTERIAL#
L5 67 S L3 AND (BACTERIA OR BACTERIAL#)
L6 49 DUP REM L5 (18 DUPLICATES REMOVED)
L7 48 S L6 NOT PY>1998

FILE 'CAPLUS' ENTERED AT 11:34:23 ON 12 FEB 2002

E NILSSON D/AU 25
L8 5 S (E3 OR E4 OR E6 OR E7 OR E8) AND (BACTERIOPHAGE)
E JANZEN T/AU 25
L9 2 S (E3 OR E4) AND (BACTERIOPHAGE)

=>

YOU HAVE REQUESTED DATA FROM 48 ANSWERS - CONTINUE? Y/(N):y

L7 ANSWER 1 OF 48 MEDLINE
 ACCESSION NUMBER: 1998307384 MEDLINE
 DOCUMENT NUMBER: 98307384 PubMed ID: 9645477
 TITLE: Efficient introduction of alkene functionality into proteins in vivo.
 AUTHOR: van Hest J C; Tirrell D A
 CORPORATE SOURCE: Department of Polymer Science and Engineering, University of Massachusetts, Amherst 01003, USA.
 SOURCE: FEBS LETTERS, (1998 May 22) 428 (1-2) 68-70.
 Journal code: EUH; 0155157. ISSN: 0014-5793.
 PUB. COUNTRY: Netherlands
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199807
 ENTRY DATE: Entered STN: 19980723
 Last Updated on STN: 19980723
 Entered Medline: 19980714

AB The methionine analogue 2-amino-5-hexenoic acid (homoallylglycine, Hag) can be utilized by Escherichia coli in the initiation and elongation steps of protein biosynthesis. Use of an E. coli methionine auxotroph and Hag-supplemented medium resulted in replacement of ca. 85% of the methionine residues in mouse dihydrofolate reductase expressed under control of a bacteriophage T5 promoter. N-terminal sequencing indicated 92+/-5% occupancy of the initiator site by Hag. The vinyl function of Hag remains intact in the purified protein and suggests new chemistries for modification of natural and artificial proteins prepared in bacterial hosts.

L7 ANSWER 2 OF 48 MEDLINE
 ACCESSION NUMBER: 97113445 MEDLINE
 DOCUMENT NUMBER: 97113445 PubMed ID: 8955305
 TITLE: Construction of a chromosome map for the phage group II Staphylococcus aureus Ps55.
 AUTHOR: Bannantine J P; Pattee P A
 CORPORATE SOURCE: Department of Microbiology, Immunology, and Preventive Medicine, Iowa State University, Ames 50011, USA..
 john_bannantine@nih.gov
 SOURCE: JOURNAL OF BACTERIOLOGY, (1996 Dec) 178 (23) 6842-8.
 Journal code: HH3; 2985120R. ISSN: 0021-9193.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199701
 ENTRY DATE: Entered STN: 19970219
 Last Updated on STN: 19990129
 Entered Medline: 19970128

AB The genome size and a partial physical and genetic map have been defined for the phage group II Staphylococcus aureus Ps55. The genome size was estimated to be 2,771 kb by pulsed-field gel electrophoresis (PFGE) using the restriction enzymes SmaI, CspI, and SgrAI. The Ps55 chromosome map was constructed by transduction of auxotrophic and cryptic transposon insertions, with known genetic and physical locations in S. aureus NCTC 8325, into the Ps55 background. PFGE and DNA hybridization analysis were used to detect the location of the transposon in Ps55. Ps55 restriction fragments were then ordered on the basis of genetic conservation between the two strains. Cloned DNA probes containing the lactose operon (lac) and genes encoding staphylococcal protein A (spa), gamma hemolysin (hlg), and coagulase (coa) were also located on the map by PFGE and hybridization analysis. This methodology enabled a direct comparison of chromosomal organization between NCTC 8325 and Ps55 strains. The chromosome size, gene order, and some of the restriction sites are conserved between the two phage group strains.

L7 ANSWER 3 OF 48 MEDLINE
 ACCESSION NUMBER: 95186989 MEDLINE
 DOCUMENT NUMBER: 95186989 PubMed ID: 7881274
 TITLE: A novel method for selective isotope labeling of bacterially expressed proteins.
 AUTHOR: Lee K M; Androphy E J; Baleja J D
 CORPORATE SOURCE: Department of Biochemistry, Tufts University School of Medicine, Boston, MA 02111.
 SOURCE: JOURNAL OF BIOMOLECULAR NMR, (1995 Jan) 5 (1) 93-6.
 Journal code: BJM; 9110829. ISSN: 0925-2738.
 PUB. COUNTRY: Netherlands
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals

ENTRY MONTH: 199504
 ENTRY DATE: Entered STN: 19950425
 Last Updated on STN: 19990129
 Entered Medline: 19950411

AB A novel method for isotope labeling in selected amino acids is presented for use with the T7 RNA polymerase system. The protocol is illustrated with the DNA-binding domain from the E2 protein of bovine papillomavirus, BPV-1. On addition of rifampicin, protein expression occurs exclusively from the gene controlled by the T7 promoter. Since the bacteria are now dedicated to the production of E2 protein, labeling with specific amino acids is efficiently performed. For example, 10 mg/l of 15N-labeled phenylalanine is shown to be sufficient for incorporation of the label, without scrambling, and without the use of an auxotrophic strain.

L7 ANSWER 4 OF 48 MEDLINE
 ACCESSION NUMBER: 93106943 MEDLINE
 DOCUMENT NUMBER: 93106943 PubMed ID: 8093236
 TITLE: The Escherichia coli mutant requiring D-glutamic acid is the result of mutations in two distinct genetic loci.
 AUTHOR: Dougherty T J; Thanassi J A; Pucci M J
 CORPORATE SOURCE: Department of Microbiology, Bristol-Myers Squibb Company
 Pharmaceutical Research Institute, Wallingford, Connecticut
 06492-7660.
 SOURCE: JOURNAL OF BACTERIOLOGY, (1993 Jan) 175 (1) 111-6.
 Journal code: HH3; 2985120R. ISSN: 0021-9193.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199301
 ENTRY DATE: Entered STN: 19930212
 Last Updated on STN: 19990129
 Entered Medline: 19930127

AB D-Glutamic acid is an essential component of bacterial cell wall peptidoglycan in both gram-positive and gram-negative bacteria. Very little is known concerning the genetics and biochemistry of D-glutamate production in most bacteria, including Escherichia coli. Evidence is presented in this report for the roles of two distinct genes in E. coli WM335, a strain which is auxotrophic for D-glutamate. The first gene, which restores D-glutamate independence in WM335, was mapped, cloned, and sequenced. This gene, designated dga, is a previously reported open reading frame, located at 89.8 min on the E. coli map. The second gene, gltS, is located at 82 min. gltS encodes a protein that is involved in the transport of D- and L-glutamic acid into E. coli, and the gltS gene of WM335 was found to contain two missense mutations. To construct D-glutamate auxotrophs, it is necessary to transfer sequentially the mutated gltS locus, and then the mutated dga locus into the recipient. The sequences of the mutant forms of both dga and gltS are also presented.

L7 ANSWER 5 OF 48 MEDLINE
 ACCESSION NUMBER: 92392276 MEDLINE
 DOCUMENT NUMBER: 92392276 PubMed ID: 1387788
 TITLE: A large decrease in heat-shock-induced proteolysis after tryptophan starvation leads to increased expression of phage lambda lysozyme cloned in Escherichia coli.
 AUTHOR: Soumillion P; Fastrez J
 CORPORATE SOURCE: Laboratoire de Biochimie Physique et des Biopolymeres,
 Universite Catholique de Louvain, Belgium.
 SOURCE: BIOCHEMICAL JOURNAL, (1992 Aug 15) 286 (Pt 1) 187-91.
 Journal code: 9YO; 2984726R. ISSN: 0264-6021.
 PUB. COUNTRY: ENGLAND: United Kingdom
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199210
 ENTRY DATE: Entered STN: 19921023
 Last Updated on STN: 19980206
 Entered Medline: 19921006

AB The R gene coding for phage lambda lysozyme (lambda L), cloned under the control of the PL promoter on a multicopy vector, is expressed in an Escherichia coli strain auxotrophic for tryptophan. Induction by a thermal shift after tryptophan supplementation in a culture initially brought into stationary phase by tryptophan starvation leads to highly increased expression. A thermally unstable mutant protein, difficult to obtain under standard conditions, can be easily produced by post-stationary-phase expression. It is shown that this is due to a drastic decrease in the heat-shock-induced proteolysis normally observed on thermal induction. These data are discussed in relation to our present knowledge of stringent and heat-shock responses.

L7 ANSWER 6 OF 48

MEDLINE

ACCESSION NUMBER: 92121881 MEDLINE
 DOCUMENT NUMBER: 92121881 PubMed ID: 1663151
 TITLE: Characterization of lip expression in Salmonella typhimurium: analysis of lip::lac operon fusions.
 AUTHOR: Smith R L; Pelley J W; Jeter R M
 CORPORATE SOURCE: Department of Biological Sciences, Texas Tech University, Lubbock 79409.
 SOURCE: JOURNAL OF GENERAL MICROBIOLOGY, (1991 Oct) 137 (Pt 10) 2307-12.
 Journal code: I87; 0375371. ISSN: 0022-1287.
 PUB. COUNTRY: ENGLAND: United Kingdom
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199202
 ENTRY DATE: Entered STN: 19920315
 Last Updated on STN: 19980206
 Entered Medline: 19920225

AB Strains of Salmonella typhimurium which have an auxotrophic requirement for lipoic acid were isolated by mutagenesis with the transposable element Mu dJ. The chromosomal location of these insertion mutations was determined to be at 14 map units by bacteriophage P22-mediated cotransduction. The lip gene is transcribed in the clockwise direction relative to the S. typhimurium genetic map. Strains with lip::lac operon fusions were used to characterize the transcriptional activity of the lip promoter. Transcription of the lip gene is not regulated by catabolite repression or lipoic acid concentration. The data indicate that the lip gene product is expressed constitutively at a low level.

L7 ANSWER 7 OF 48

MEDLINE

ACCESSION NUMBER: 91100289 MEDLINE
 DOCUMENT NUMBER: 91100289 PubMed ID: 1702779
 TITLE: Identification and characterization of dppA, an Escherichia coli gene encoding a periplasmic dipeptide transport protein.
 AUTHOR: Olson E R; Dunyak D S; Jurss L M; Poorman R A
 CORPORATE SOURCE: Molecular Biology Research, Upjohn Company, Kalamazoo, Michigan 49007.
 SOURCE: JOURNAL OF BACTERIOLOGY, (1991 Jan) 173 (1) 234-44.
 Journal code: HH3; 2985120R. ISSN: 0021-9193.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-M35045
 ENTRY MONTH: 199102
 ENTRY DATE: Entered STN: 19910329
 Last Updated on STN: 19960129
 Entered Medline: 19910220

AB We describe the isolation and analysis of an Escherichia coli gene, dppA, and its role in dipeptide transport. dppA maps near min 79 and encodes a protein (DppA) that has regions of amino acid similarity with a peptide-binding protein from Salmonella typhimurium (OppA). Like OppA, DppA is found in the periplasmic space and thus is most likely a dipeptide-binding protein. Insertional inactivation of dppA results in the inability of a proline auxotroph to utilize Pro-Gly as a proline source. dppA-dependent Pro-Gly utilization does not require any of the three major proline transport systems, demonstrating that DppA is not simply a dipeptidase. An in vivo competition assay was used to show that DppA is probably involved in the transport of dipeptides other than Pro-Gly. Transcription of dppA is repressed by the presence of casamino acids, suggesting that the cell alters its dipeptide transport capabilities in response to an environmental signal.

L7 ANSWER 8 OF 48

MEDLINE

ACCESSION NUMBER: 90330624 MEDLINE
 DOCUMENT NUMBER: 90330624 PubMed ID: 1973930
 TITLE: Nucleotide sequence of Escherichia coli asnB and deduced amino acid sequence of asparagine synthetase B.
 AUTHOR: Scofield M A; Lewis W S; Schuster S M
 CORPORATE SOURCE: Department of Chemistry, University of Nebraska, Lincoln 68588.
 CONTRACT NUMBER: CA28725 (NCI)
 P41RR01685 (NCRR)
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 Aug 5) 265 (22) 12895-902.
 Journal code: HIV; 2985121R. ISSN: 0021-9258.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-J05554
 ENTRY MONTH: 199009
 ENTRY DATE: Entered STN: 19901012
 Last Updated on STN: 19950206
 Entered Medline: 19900904

AB The Escherichia coli asparagine synthetase B gene (asnB) has been cloned into a temperature-sensitive, low copy plasmid, pOU71, as shown by the complementation of an E. coli asparagine auxotroph, E. coli JE6279. The nucleotide sequence of asnB and the flanking sequences were determined. The proposed coding region for the gene is 1662 nucleotides in length, and the deduced amino acid sequence of the coding region results in a protein that has a molecular weight of 62,666 and contains 554 amino acids. A promoter region is identified based on the transcription start site that was determined by primer extension experiments. Homology studies of the asnB protein sequence with the human asparagine synthetase and E. coli asparagine synthetase A protein show that there is a high degree of homology with only the human asparagine synthetase. A purF type glutamine amide transfer domain was identified upon inspection of the amino-terminal amino acid sequence of the asparagine synthetase B protein.

L7 ANSWER 9 OF 48 MEDLINE
 ACCESSION NUMBER: 87089965 MEDLINE
 DOCUMENT NUMBER: 87089965 PubMed ID: 3842739
 TITLE: [Acetobacter methanolicus--a new organism for genetic studies].
 Acetobacter methanolicus--novyi ob''ekt geneticheskikh issledovaniy.
 AUTHOR: Sattler K; Wunsche L; Engel J; Babel W
 SOURCE: MOLEKULIARNAYA GENETIKA, MIKROBIOLOGIA, I VIRUSOLOGA, (1985 Jan) (1) 33-4.
 Journal code: NMJ; 9315607. ISSN: 0208-0613.
 PUB. COUNTRY: USSR
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: Russian
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 198702
 ENTRY DATE: Entered STN: 19900302
 Last Updated on STN: 19900302
 Entered Medline: 19870205

AB A new bacterial strain is described belonging to Acetobacter methanolicus species. It is of industrial value as a producer of protein and methanol products. The strain is acidophile and this feature comprises a conspicuous technological advantage. The results of bacteriophage and cell interactions are reported. They might be potentially useful for elaboration of the transduction technique for the strain. The collection of mutants was obtained including those utilizing methanol, having auxotrophic markers as well as streptomycin and rifampicin resistances. The transfer of plasmids RSF1010 and R68 to Acetobacter methanolicus from other bacteria has been demonstrated.

L7 ANSWER 10 OF 48 MEDLINE
 ACCESSION NUMBER: 77190336 MEDLINE
 DOCUMENT NUMBER: 77190336 PubMed ID: 325229
 TITLE: Effects of temperature and host cell genetic characteristics on the replication of the lipid-containing bacteriophage PR4 in Escherichia coli.
 AUTHOR: Sands J A; Auperin D
 SOURCE: JOURNAL OF VIROLOGY, (1977 May) 22 (2) 315-20.
 Journal code: KCV; 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 197707
 ENTRY DATE: Entered STN: 19900314
 Last Updated on STN: 19980206
 Entered Medline: 19770718

AB The lipid-containing bacteriophage PR4 is of special interest because it can replicate in various gram-negative bacteria, including Escherichia coli, that carry one of a group of drug resistance plasmids. PR4 grown in E. coli strain PS2R contains about 10% lipid by weight, with the negatively charged phospholipid phosphatidylglycerol being the most abundant lipid in the virion. We now report the following. (i) PR4 attaches to E. coli with an attachment rate constant of K_a approximately $6.2 \times 10^{(-10)}$ ml/min, which is about twice that of the enveloped phage phi6 (to Pseudomonas phaseolicola), but a factor of 5 less than that of phage PM2 (to Pseudomonas BAL-31). (ii) Use of an E. coli glycerol auxotroph indicated that a normal amount of PR4

replication occurs only if glycerol starvation (inhibition of all phospholipid synthesis) begins no earlier than about halfway through the lytic cycle. (iii) Use of an E. coli fatty acid synthesis temperature-sensitive mutant and an E. coli phosphatidylethanolamine synthesis temperature-sensitive mutant indicate that PR4 replication can occur in the absence of either normal fatty acid synthesis or normal phospholipid synthesis if the infection takes place prior to the termination of overall cell growth and the onset of cell death, (iv) Whereas PR4 burst size in nutrient media at 30 degrees C to 42°C is about 40, the burst size at 20 degrees C is less than 3, Temperature-shift experiments show that the temperature late in infection determines the burst size.

L7 ANSWER 11 OF 48 MEDLINE
 ACCESSION NUMBER: 72151570 MEDLINE
 DOCUMENT NUMBER: 72151570 PubMed ID: 4552550
 TITLE: Polyamines in the synthesis of bacteriophage deoxyribonucleic acid. II. Requirement for polyamines in T4 infection of a polyamine auxotroph
 AUTHOR: Dion A S; Cohen S S
 SOURCE: JOURNAL OF VIROLOGY, (1972 Mar) 9 (3) 423-30.
 Journal code: KCV; 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 197206
 ENTRY DATE: Entered STN: 19900310
 Last Updated on STN: 19900310
 Entered Medline: 19720608

L7 ANSWER 12 OF 48 CAPLUS COPYRIGHT 2002 ACS
 ACCESSION NUMBER: 1997:488123 CAPLUS
 DOCUMENT NUMBER: 127:118262
 TITLE: Auxotrophic mutant starter and feeder cells in cross-feeding system with reversibly noninfective modified lambdoid bacteriophage to produce colony containing starter cells that excrete a desired protein
 INVENTOR(S): Ray, Bryan L.; Lin, Edmund C. C.; Crea, Roberto
 PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA
 SOURCE: U.S., 34 pp. Cont.-in-part of U.S. 5,348,872.
 CODEN: USXXAM
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 3
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5646030	A	19970708	US 1994-294386	19940823
US 5348872	A	19940920	US 1992-991115	19921216
WO 9606164	A1	19960229	WO 1995-US10224	19950810
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9533635	A1	19960314	AU 1995-33635	19950810
PRIORITY APPLN. INFO.:				
			US 1990-541895	19900621
			US 1992-856876	19920324
			US 1992-991115	19921216
			US 1994-294386	19940823
			WO 1995-US10224	19950810

AB Disclosed is a method for isolating a mutant cell that excretes a desired compd. The method includes culturing a plurality of auxotrophic pretreated starter cells and auxotrophic feeder cells in the presence of a reversibly noninfective, modified lambdoid bacteriophage. If the treated starter cell produces the desired compd., the bacteriophage will be rendered infective and infect the feeder cell. The feeder cell, in turn, will excrete a metabolite required by the starter cell and the starter cell will excrete a metabolite required by the feeder cell, enabling the cells to cross-feed, grow, and produce a colony contg. a starter cell which produces the desired compd. The method takes advantage that lambdoid bacteriophage having a target mol. peptide linked to the glycoprotein gpV gene can be successfully assembled in vivo such that the target mol. is displayed on the outer surface of the bacteriophage. The method is illustrated by using the chem. modified bacteriophage .lambda. for (1) screening treated bacteria for prodn. of ciliary neurotrophic factor or interleukin-1.beta. converting enzyme, (2) screening genetically engineered yeast producing erythropoietin, (3) screening Corynebacterium glutamicum for prodn. of staphylococcal nuclease, (4) isolation of a transformed human Bj-cell

producing antibodies to hepatitis C virus, and (5) isolation of digitalis lanata plant cells producing quinine.

L7 ANSWER 13 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:321410 CAPLUS
DOCUMENT NUMBER: 124:334859
TITLE: Auxotrophic mutant starter and feeder cells
in cross-feeding system with reversibly noninfective
modified lambdoid bacteriophage to produce
colony containing starter cells that excrete a desired
protein
INVENTOR(S): Ray, Bryan L.; Lin, Edmund C. C.; Crea, Roberto
PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 88 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9606164	A1	19960229	WO 1995-US10224	19950810
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5646030	A	19970708	US 1994-294386	19940823
AU 9533635	A1	19960314	AU 1995-33635	19950810
PRIORITY APPLN. INFO.:			US 1994-294386	19940823
			US 1990-541895	19900621
			US 1992-856876	19920324
			US 1992-991115	19921216
			WO 1995-US10224	19950810

AB Disclosed is a method for isolating a mutant cell that excretes a desired compd. The method includes culturing a plurality of auxotrophic pretreated starter cells and auxotrophic feeder cells in the presence of a reversibly noninfective, modified lambdoid bacteriophage. If the treated starter cell produces the desired compd., the bacteriophage will be rendered infective and infect the feeder cell. The feeder cell, in turn, will excrete a metabolite required by the starter cell and the starter cell will excrete a metabolite required by the feeder cell, enabling the cells to cross-feed, grow, and produce a colony contg. a starter cell which produces the desired compd.

L7 ANSWER 14 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:650967 CAPLUS
DOCUMENT NUMBER: 121:250967
TITLE: Maturation of bacteriophage 9NA DNA is
influenced by the fatty acid composition of
the host cell membrane
AUTHOR(S): Goyal, Ruby; Sasane, Grace; Majumdar, Sabita;
Chakravorty, Maharani
CORPORATE SOURCE: Institute Medical Sciences, Banaras Hindu University,
Varanasi, 221 005, India
SOURCE: J. Biosci. (Bangalore, India) (1994), 19(2), 183-92
CODEN: JOBSDN; ISSN: 0250-5991
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The fatty acid compn. of the membrane of the conditional auxotroph fabB2 can be altered by allowing the cells to grow at non-permissive temp. (37.degree.C) in the presence of a cis-unsatd. fatty acid. Phage 9NA, a virulent phage of Salmonella typhimurium, can not multiply in fabB2. Synthesis and maturation of the phage DNA are differentially affected by variation in the fatty acid compn. of the cell membrane. The replicating DNA assoc. with the membrane complex, the site of DNA synthesis. The assocn. is comparatively weak in oleic, elaidic, palmitoleic, palmitelaidic and linolelaidic acid enriched cells. When the cells are grown in the presence of palmitoleic acid, a large pool of concatemeric phage DNA accumulates in the cytoplasm within 10 min of infection. The conversion of concatemeric DNA to monomeric one i.e., mature phage length DNA, is inhibited in such cells. The presence of concatemeric DNA can be visualized by electron microscope. Such a situation is not obsd. when the cells are grown in media supplemented with other types of unsatd. fatty acids. The mechanism by which the host cell membrane lipid controls phage development is yet to be worked out.

L7 ANSWER 15 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:645308 CAPLUS
DOCUMENT NUMBER: 119:245308
TITLE: Phage Acml-mediated transduction in the facultatively
methanol-utilizing Acetobacter methanolicus MB 58/4

AUTHOR(S): Kiesel, Baerbel; Wuensche, Lothar
 CORPORATE SOURCE: Cent. Environ. Res. Leipzig-Halle GmbH, Leipzig, 04318, Germany
 SOURCE: J. Gen. Virol. (1993), 74(9), 1741-5
 CODEN: JGVIAY; ISSN: 0022-1317
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Phage Acml, generally virulent for the acidophilic facultatively methanol-utilizing strain of *Acetobacter methanolicus* MB 58.4, is also capable of lysogenizing its host strain at a low rate. Using amino acid-auxotrophic mutants of *A. methanolicus* MB 58/4 as recipient strains, transduction of His, Leu and Tyr markers could be demonstrated in this system. The ability to prep. transducing lysates by propagation of phage Acml on the prototrophic donor strain *A. methanolicus* MB 58/4, the transduction of three different markers as well as the efficiency of transduction, and the occurrence of permutations in the phage genome indicate that phage Acml mediates generalized transduction. Phage Acml might be a useful tool in genetic studies of methylophilic *A. methanolicus*.

L7 ANSWER 16 OF 48 CAPLUS COPYRIGHT 2002 ACS
 ACCESSION NUMBER: 1993:120215 CAPLUS
 DOCUMENT NUMBER: 118:120215
 TITLE: Spectral enhancement of proteins: Biological incorporation and fluorescence characterization of 5-hydroxytryptophan in bacteriophage .lambda. cI repressor
 AUTHOR(S): Ross, J. B. Alexander; Senear, Donald F.; Waxman, Evan; Kombo, Bamenga B.; Rusinova, Elena; Huang, Yao Te; Laws, William R.; Hasselbacher, C. A.
 CORPORATE SOURCE: Dep. Biochem., Mt. Sinai Sch. Med., New York, NY, 10029, USA
 SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1992), 89(24), 12023-7
 CODEN: PNASA6; ISSN: 0027-8424
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB A tryptophan-requiring *Escherichia coli* auxotroph was used to replace the 3 tryptophan residues of .lambda. cI repressor with 5-hydroxy-L-tryptophan (5-OHTrp). By using a nonleaky promoter, >95% replacement of tryptophan in the repressor was achieved. The absorbance and fluorescence properties of 5-OHTrp-.lambda. cI are clearly distinct from .lambda. cI repressor, and the fluorescence of 5-OHTrp-.lambda. cI repressor can be obsd. selectively in the presence of exogenous tryptophan. The 5-OHTrp-.lambda. cI repressor functional properties, as assessed by measurement of binding consts. for self-assocn. and for assocn. to operator DNA, and structural properties, as assessed by fluorescence, are indistinguishable from the native repressor. Based on these results, it is anticipated that the availability of spectrally enhanced proteins will significantly enhance the utility of both fluorescence and phosphorescence spectroscopies to study protein structure and function in complex interacting systems.

L7 ANSWER 17 OF 48 CAPLUS COPYRIGHT 2002 ACS
 ACCESSION NUMBER: 1989:436545 CAPLUS
 DOCUMENT NUMBER: 111:36545
 TITLE: Abortive infection of the virulent phage 9NA in a fatty acid auxotroph of *Salmonella typhimurium*: effect of fatty acid supplementation
 AUTHOR(S): Goyal, Ruby; Chakravorty, Maharani
 CORPORATE SOURCE: Inst. Med. Sci., Banaras Hindu Univ., Varanasi, 221 005, India
 SOURCE: Biochem. Biophys. Res. Commun. (1989), 161(2), 923-30
 CODEN: BBRCA9; ISSN: 0006-291X
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB A conditional (temp. sensitive) fatty acid biosynthetic mutant (fabB2) of *S. typhimurium* does not support the development of the virulent bacteriophage 9NA even at permissive temp. (30.degree.). A limited amt. of phage DNA synthesis takes place at this temp. When the fatty acid compn. of the host membrane is altered by growing the cells at 37.degree. in the presence of exogenous unsatd. fatty acid, differential expression of phage genes was obsd. Phage specific lysozyme is induced when the cultures are supplemented with elaidic, palmitelaidic, linoleic, and linolelaidic acids but not with oleic and palmitoleic acids. However, in no case were infective particles produced. Under conditions where no lysozyme is synthesized the infected cells increase in length and become filamentous.

L7 ANSWER 18 OF 48 CAPLUS COPYRIGHT 2002 ACS
 ACCESSION NUMBER: 1988:125912 CAPLUS

DOCUMENT NUMBER: 108:125912
 TITLE: Non-radioactive, single-stranded, labelled DNA probe, method for its production, and method of detecting a target nucleotide sequence using this probe
 INVENTOR(S): Avrameas, Stratis; Sakamoto, Hiroshi; Traincard, Francois; Vo Quang Tuyen; Guesdon, Jean Luc; Ter Nynck, Therese
 PATENT ASSIGNEE(S): Institut Pasteur, Fr.; Centre National de la Recherche Scientifique
 SOURCE: Eur. Pat. Appl., 9 pp.
 CODEN: EPXXDW
 DOCUMENT TYPE: Patent
 LANGUAGE: French
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 242264	A1	19871021	EP 1987-400747	19870403
R: AT, BE, CH, DE, ES, GB, GR, IT, LI, LU, NL, SE				
FR 2596773	A1	19871009	FR 1986-4913	19860404
FR 2596773	B1	19880624		
JP 62253385	A2	19871105	JP 1987-83685	19870403
US 4978749	A	19901218	US 1989-378507	19890711
PRIORITY APPLN. INFO.:			FR 1986-4913	19860404
			US 1987-33193	19870402

AB A nonradioactive, single-stranded, labeled (e.g. with halogenated nucleotide bases) DNA hybridization probe is prepd. by infection of a microbial auxotroph with a bacteriophage (e.g. M 13) and growing the microorganism in medium contg. a halogenated nucleic acid base. Escherichia coli TUC0701 (a thymine-requiring mutant) was infected with M 13 and grown in a medium contg. 5-bromouracil. At least 10 .mu.g labeled DNA/mL culture was produced. The probe was isolated both from the cells and from the encapsidated phage in the medium.

L7 ANSWER 19 OF 48 CAPLUS COPYRIGHT 2002 ACS
 ACCESSION NUMBER: 1987:552772 CAPLUS
 DOCUMENT NUMBER: 107:152772
 TITLE: Acetobacter methanolicus: a new object of genetic research
 AUTHOR(S): Sattler, K.; Wunsche, L.; Engel, J.; Babel, W.
 CORPORATE SOURCE: Inst. Tekh. Chem., Leipzig, Ger. Dem. Rep.
 SOURCE: Mol. Genet., Mikrobiol. Virusol. (1985), (1), 33-4
 CODEN: MGMVDU
 DOCUMENT TYPE: Journal
 LANGUAGE: Russian
 AB A new bacterial strain is described belonging to the Acetobacter methanolicus species. It is of industrial value as a producer of protein and methanol products. The strain is acidophilic, and this feature comprises a conspicuous technol. advantage. The results of bacteriophage and cell interactions are reported. The collection of mutants was obtained including those utilizing methanol, having auxotrophic markers as well as streptomycin and rifampicin resistances. The transfer of plasmids RSF1010 and R68 to A. methanolicus from other bacteria has been demonstrated.

L7 ANSWER 20 OF 48 CAPLUS COPYRIGHT 2002 ACS
 ACCESSION NUMBER: 1986:624581 CAPLUS
 DOCUMENT NUMBER: 105:224581
 TITLE: Integration of a gene into a chromosome and controllable excision from it
 INVENTOR(S): Balakrishnan, Ramaswamy; Backman, Keith C.
 PATENT ASSIGNEE(S): BioTechnica International, Inc., USA; Heinz, H. J., Co.
 SOURCE: Eur. Pat. Appl., 31 pp.
 CODEN: EPXXDW
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 191643	A2	19860820	EP 1986-301000	19860213
EP 191643	A3	19881102		
EP 191643	B1	19920513		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
US 4743546	A	19880510	US 1985-701091	19850213
CA 1339799	A1	19980407	CA 1986-501705	19860212
JP 61247389	A2	19861104	JP 1986-29857	19860213
AT 76103	E	19920515	AT 1986-301000	19860213

PRIORITY APPLN. INFO.:

US 1985-701091 19850213
EP 1986-301000 19860213

AB A method for controlling cell growth comprises integration of an excision vector into the chromosome of a bacterial cell which is auxotrophic, where the excision vector contains (1) the gene encoding the nutrient needed for cell growth, (2) a phage-derived DNA sequence encoding an attachment site (e.g. att) and excision enzymes (e.g. int, xis), and (3) a gene encoding a regulator protein (e.g. cI⁸⁵⁷) which causes excision of the vector from the host chromosome in response to an external stimulus (e.g. heat shock). Thus, the cells can be grown to a desired d. in the presence of the integrated vector, at which point their growth can be halted using the external stimulus. Cessation of a cell function or growth is useful in microbial fermn. of a desired product.

L7 ANSWER 21 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1982:82422 CAPLUS
DOCUMENT NUMBER: 96:82422
TITLE: Direction of bacteriophage .lambda. DNA replication in a thymine requiring Escherichia coli K-12 strain. Effect of thymidine concentration
AUTHOR(S): Valenzuela, Manuel S.; Inman, Ross B.
CORPORATE SOURCE: Dep. Biochem., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: Nucleic Acids Res. (1981), 9(24), 6975-84
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The direction of replication was established for the 1st round of bacteriophage .lambda. DNA replication in thymine-requiring E. coli K-12 cells exposed to different concns. of thymidine. A dramatic decrease in the proportion of bidirectionally replicating mols. followed a decrease in the concn. of thymidine. Moreover, the rightward mode of replication appears to be exclusively favored in unidirectionally replicating mols. found at low concns. of thymidine.

L7 ANSWER 22 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1982:46841 CAPLUS
DOCUMENT NUMBER: 96:46841
TITLE: Transcription regulation of colicin Ib synthesis
AUTHOR(S): Pugsley, Anthony P.
CORPORATE SOURCE: Biocent., Univ. Basle, Basel, CH-4056, Switz.
SOURCE: MGG, Mol. Gen. Genet. (1981), 183(3), 522-7
CODEN: MGGEAE; ISSN: 0026-8925
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bacteriophage Mud was used to bring the transcription of the gene for .beta.-galactosidase (lacZ) under the control of the promoter of the structural gene for colicin Ib [37217-78-0] (cia) on a deriv. of the Col plasmid ColIb.P9. Transcription of this fusion operon was stimulated by agents which damaged cellular DNA (mitomycin C, bleomycin, and colicin E2). Increased transcription of the cia-lacZ operon could be detected within 13 min of the addn. of these agents. In a strain bearing the tif-1 (recA441) mutation, constitutive expression of the SOS DNA repair system at 42.degree. also increased transcription of the cia-lacZ operon. Transcription of the cia-lacZ operon was also stimulated by inhibition of DNA gyrase activity with nalidixic acid but not with novobiocin. Transitory inhibition of protein synthesis with chloramphenicol or by proline starvation of a proline auxotroph did not stimulate cia-lacZ transcription. Transcription of the cia-lacZ operon was substantially reduced in the presence of a recA mutation, but was largely unaffected by a mutation in recB affecting the RecBC DNase or by catabolite repression. Control expts. in which the prodn. of colicin Ib was measured confirmed that the expts. with the fusion operon gave an accurate indication as to the activity of the wild type cia gene except for the effect of catabolite repression, where .ltoreq.90% redn. in colicin Ib prodn. was obsd. with strains carrying mutant crp or cya alleles. Thus, there is considerable similarity between the regulatory systems controlling prodn. of colicins and the repressor-dependent regulation of lambdoid prophage induction.

L7 ANSWER 23 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1978:85859 CAPLUS
DOCUMENT NUMBER: 88:85859
TITLE: Production of a functional eukaryotic enzyme in Escherichia coli: Cloning and expression of the yeast structural gene for imidazoleglycerolphosphate dehydratase (his3)
AUTHOR(S): Struhl, Kevin; Davis, Ronald W.
CORPORATE SOURCE: Dep. Biochem., Stanford Univ. Sch. Med., Stanford, Calif., USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1977), 74(12), 5255-9
CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal
LANGUAGE: English

AB A cloned segment of yeast DNA contg. the structural gene for imidazoleglycerol phosphate dehydratase (EC 4.2.1.19) is transcribed and translated in *E. coli* with sufficient fidelity to produce functional enzyme. This segment of yeast DNA was isolated as a viable mol. hybrid of bacteriophage .lambda. (.lambda.gt-Sc2601) which complements a nonrevertible *hisB* auxotroph of *E. coli* lacking dehydratase activity. The equiv. segments of DNA cloned from 2 independent *his3* mutants of yeast lacking imidazoleglycerol phosphate dehydratase activity do not complement the *hisB* auxotroph. The 2 nonfunctional *his3* alleles cloned in bacteriophage .lambda. can be recombined in *E. coli* to generate a hybrid phage which complements the *hisB* auxotroph. The dehydratase activity produced in *E. coli* by the cloned segment of yeast DNA strongly resembles the activity found in yeast.

L7 ANSWER 24 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1977:548520 CAPLUS
DOCUMENT NUMBER: 87:148520
TITLE: Changes in macromolecular synthesis in *Xanthomonas oryzae* infected with bacteriophage XP-12
AUTHOR(S): Ehrlich, Melanie; Lin, Fu-Hsuing; Ehrlich, Kenneth; Brown, Susan L.; Mayo, John A.
CORPORATE SOURCE: Dep. Biochem., Tulane Med. Sch., New Orleans, La., USA
SOURCE: J. Virol. (1977), 23(3), 517-23
CODEN: JOVIAM
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Phage XP-12, which has complete substitution of the cytosine residues in its DNA with 5-methylcytosine residues, inhibited incorporation of uracil into host DNA and RNA during the latent period. This apparent inhibition of host macromol. synthesis was not accompanied by extensive degradn. of the host chromosome. Phage DNA synthesis in infected cells occurred at a faster rate than host DNA synthesis in analogous uninfected cells. However, phage DNA synthesis could not be accurately monitored by incorporation of thymidine-methyl-3H into DNA because, soon after infection, there was a marked inhibition of utilization of exogenous thymidine for DNA synthesis. Phage infection conferred upon a thymine auxotrophic host the ability to synthesize thymine nucleotides for phage DNA synthesis. A phage-induced thymidylate synthetase activity may be partially responsible for the inhibition of thymidine incorporation.

L7 ANSWER 25 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1977:136172 CAPLUS
DOCUMENT NUMBER: 86:136172
TITLE: Structure and synthesis of a lipid-containing bacteriophage. Effects of lipids containing cis or trans fatty acids on the reconstitution of bacteriophage PM2
AUTHOR(S): Tsukagoshi, Norihiro; Schaefer, Rolf; Franklin, Richard M.
CORPORATE SOURCE: Biocent., Univ. Basel, Basel, Switz.
SOURCE: Eur. J. Biochem. (1977), 73(2), 469-76
CODEN: EJBCAI
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Infectious PM2 virus particles could be reconstituted in vitro from a mixt. of nucleocapsid, phospholipids contg. cis fatty acids, and proteins I and II. The presence or absence of acyl phosphatidylglycerol, a minor lipid component of the virion, did not affect the reconstitution of infectious particles, even though it was incorporated into the particles when present. When phosphatidylglycerol was completely replaced by acyl phosphatidylglycerol in the reconstitution mixt., no infectious particles were formed. Lipids contg. either cis or trans fatty acids were also used for reconstitution in vitro of the lipid-contg. bacteriophage PM2. Regardless of the ratio of phosphatidylglycerol to phosphatidylethanolamine in the reconstitution mixt., infectious particles were formed and had almost the same phospholipid compn. when lipids contg. cis-palmitoleic acid were used; no infectious particles were obtained when lipids contg. trans-palmitoleic acid were used. In the latter case, virus-like particles were, however, formed. Reconstitution particles contg. cis fatty acids were infectious when tested on wild type *Pseudomonas* BAL-31 as well as on the unsatd. fatty acid auxotroph grown in the presence of either cis- or trans-palmitoleic acid. Reconstituted particles contg. trans fatty acids were not infectious on any of these cells. When trans fatty acids as well as cis fatty acids were present in the reconstitution mixt., then there was a lower yield of infectious particles. Particles with either cis or trans fatty acids had all 4 viral proteins and adsorbed to BAL-31 host cells

in a specific manner.

L7 ANSWER 26 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1976:118390 CAPLUS
DOCUMENT NUMBER: 84:118390
TITLE: Phase transitions in the membrane of a marine bacterium, *Pseudomonas* BAL-31
AUTHOR(S): Tsukagoshi, Norihiro; Petersen, Marianne H.; Huber, Ursula; Franklin, Richard M.; Seelig, Joachim
CORPORATE SOURCE: Biozent., Univ. Basel, Basel, Switz.
SOURCE: Eur. J. Biochem. (1976), 62(2), 257-62
CODEN: EJBCAI
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An unsatd. fatty acid auxotroph, strain UFA, isolated from the marine pseudomonad *Pseudomonas* BAL-31, host cell of the lipid-contg. bacteriophage PM2, was grown in media supplemented with different unsatd. fatty acids. Under these conditions the fatty acid compn. of the cell could be altered drastically. The phase transition in the native membrane and in the extd. lipids was analyzed by ESR with a nitroxide spin probe. Membranes prepd. from strain UFA grown in cis 16:1 or trans 16:1 showed 1 transition at 9.4.degree. and 12.4.degree. resp. Extd. lipids in both cases had almost the same transition temp. as that of the intact membrane. Membranes prepd. from *Pseudomonas* BAL-31 had 1 transition at .apprx.12.degree., on the other hand there was no clear cut phase transition using extd. lipids. Replication of bacteriophage PM2 took place below the transition temp. of the membrane lipids in the case where strain UFA was grown in trans 16:1. Other cases were not studied.

L7 ANSWER 27 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1975:493520 CAPLUS
DOCUMENT NUMBER: 83:93520
TITLE: Structure and synthesis of a lipid-containing bacteriophage. XVIII. Modification of the lipid composition in bacteriophage PM2
AUTHOR(S): Tsukagoshi, Norihiro; Petersen, Marianne H.; Franklin, Richard M.
CORPORATE SOURCE: Biocent., Univ. Basel, Basel, Switz.
SOURCE: Virology (1975), 66(1), 206-16
CODEN: VIRLAX
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An unsatd. fatty acid auxotroph was isolated from *Pseudomonas* BAL-31. The fatty acid compns. of the auxotroph, wild-type cells, and virions grown on the auxotroph were detd. in media supplemented with unsatd. fatty acids. The fatty acid compn. in virions, in general, reflected that in host cells, although some differences were obsd. The phospholipid compn. in virions was very different from that in host cells and varied depending on the fatty acid used in the medium. Virions with altered lipid compn. showed almost the same heat stability and stability with respect to pH.

L7 ANSWER 28 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1974:401212 CAPLUS
DOCUMENT NUMBER: 81:1212
TITLE: Growth of ribonucleic acid bacteriophage f2 in a conditional putrescine auxotroph of *Escherichia coli*. Evidence for a polyamine role in translation
AUTHOR(S): Young, Delano V.; Srinivasan, P. R.
CORPORATE SOURCE: Coll. Physicans Surg., Columbia Univ., New York, N. Y., USA
SOURCE: J. Bacteriol. (1974), 117(3), 1280-8
CODEN: JOBAAY
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The RNA bacteriophage f2, grows poorly in a conditional putrescine auxotroph during polyamine starvation. The addn. of putrescine simultaneously with f2 enhances phage growth, shortens the latent period, and increases the burst size. This stimulation of f2 growth is reflected in higher rates of phage RNA and protein syntheses. Putrescine does not affect f2 adsorption or the penetration of its RNA, rather, in vitro assays demonstrate that putrescine-supplemented cells make more mols. of f2 replicase per incoming parental RNA than polyamine-starved cultures. The ability of polyamines to stimulate translation of a preformed messenger suggests a physiol. role for these org. cations in normal protein synthesis.

L7 ANSWER 29 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1974:57246 CAPLUS

DOCUMENT NUMBER: 80:57246
TITLE: Isolation and characterization of a generalized transducing bacteriophage for Acinetobacter
AUTHOR(S): Heman, Norma J.; Juni, Elliot
CORPORATE SOURCE: Dep. Microbiol., Univ. Michigan, Ann Arbor, Mich., USA
SOURCE: J. Virol. (1974), 13(1), 46-52
CODEN: JOVIAM
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A series of bacteriophages which grow in various strains of Acinetobacter have been isolated. One of these, phage P78, which forms turbid plaques on Acinetobacter strain 78 is specific for this particular host and fails to attack 389 other independently isolated strains of Acinetobacter. Phage P78 appears to be a temperate phage which lysogenizes its host. Various agents such as N-methyl-N'-nitro-N-nitrosoguanidine, Et2SO4, mitomycin C, and uv light are effective inducers of the lysogen. Phage lysates of wild-type cells are capable of transducing auxotrophs of strain 78 to prototrophy at frequencies ranging from 0.3 times. 10-7 to 34 times. 10-7 per plaque-forming unit adsorbed. No linkage has been detected between any of the markers studied in 2-factor crosses. Donor phage grown in a particular mutant, strain 78(arg-1), gives rise to significantly higher transduction frequencies than when phage is grown on wild-type or other auxotrophic strains. Phage P78 is rapidly adsorbed to its bacterial host, has a latent period of 25 min, and infection results in a burst size of approx. 50. Some of the phys. properties of phage P78 and its DNA are described.

L7 ANSWER 30 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1974:35005 CAPLUS
DOCUMENT NUMBER: 80:35005
TITLE: Biosynthesis of .alpha.-putrescinylothymine in bacteriophage .phi.W-14-infected Pseudomonas acidovorans
AUTHOR(S): Kelln, Rod A.; Warren, R. A. J.
CORPORATE SOURCE: Dep. Microbiol., Univ. British Columbia, Vancouver, B. C., Can.
SOURCE: J. Virol. (1973), 12(6), 1427-33
CODEN: JOVIAM
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The .alpha.-putrescinylothymine (putThy) in bacteriophage .vphi.W-14 DNA is synthesized at the mononucleotide level. It is labeled by uracil or deoxyuridine but not by thymidine, and it appears in the acid-sol. pool of infected cells before the onset of phage DNA synthesis. The methylene group at the C-5 position of the pyrimidine moiety of putThy is derived in vivo from a C1 unit. Exts. of a phage-infected thymidine auxotroph of the host, Pseudomonas acidovorans, apparently contain a phage-specific thymidylate synthetase and a phage-specific activity which forms 5-hydroxymethyl-dUMP from N5,N10-methylenetetrahydrofolate and dUMP.

L7 ANSWER 31 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1971:485621 CAPLUS
DOCUMENT NUMBER: 75:85621
TITLE: Biological action of elements of cosmic flight on lysogen bacteria Escherichia coli K-12(.lambda.) and human cells in a culture
AUTHOR(S): Zhukov-Verezhnikov, N. N.; Volkov, M. N.; Rybakov, N. I.; Maiskii, I. N.; Saksonov, P. P.; Guberniev, M. A.; Podoplelov, I. I.; Antipov, V. V.; Kozlov, V. A.; et al.
CORPORATE SOURCE: USSR
SOURCE: Kosm. Issled. (1971), 9(2), 292-9
CODEN: KOISAW
DOCUMENT TYPE: Journal
LANGUAGE: Russian

AB Lysogenic bacteria E. coli K-12(.lambda.), E. coli CA-23(D), and E. coli 200 PS with F-Lac+ episome and cultures of Hela cells were exposed to spaceflight. Changes in the bacteriophage induction and redn. in the no. carrying the F-Lac+ factor in the integrated state were obsd. The space-flight factors had no effect on the frequency of the auxotrophic mutations and induced synthesis of .beta.-galactosidase. After 4 exposures the Hela culture showed changes in cell morphol., type of growth, proliferation and antigenic properties. The obsd. mutations were apparently the result of repeated exposures during space flights.

L7 ANSWER 32 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1971:1397 CAPLUS
DOCUMENT NUMBER: 74:1397
TITLE: Production of deletions in the chromosome of Escherichia coli

AUTHOR(S): Spudich, James A.; Horn, Virginia; Yanofsky, Charles
CORPORATE SOURCE: Dep. Biol. Sci., Stanford Univ., Stanford, Calif., USA
SOURCE: J. Mol. Biol. (1970), 53(1), 49-67
CODEN: JMOBAK
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In *E. coli* B grown at 37.degree., essentially all spontaneous deletions detected by simultaneous mutation to resistance to the bacteriophage T1 and to tryptophan auxotrophy (*tonB trpdel* mutations) remove the entire *trp* operon (B-type deletions). In contrast, *tonB trpdel* mutations in *E. coli* K12 have a more random assortment of end points terminating within any of the 5 structural genes (K-type deletions) or beyond them. Chromosomal hybrids, constructed by transduction of the *trp* region from *E. coli* B into *E. coli* K12 (KB hybrids), could be divided into 2 distinct classes on the basis of their deletion patterns; some produced K-type deletions with the same relative frequency as in strain K12, while the others all produced the B-type and, without exception, at a 10- to 20-fold higher relative frequency than that obsd. in strain B. Further anal. of KB hybrids showed that the chromosomal region from *E. coli* B approx. midway between the *cysB* and *trp* operons is the region responsible for the generation of the B-type deletions characteristically found in this strain. Evidence suggested that the end points of the B-type deletions are not at fixed points in the chromosome. *TonB trp* deletions occurred in strains K12(W3110), B, and in the KB hybrids at 30, 37, and 42.degree., but in all these strains the relative frequency of deletions was reduced at 42.degree. more than 7-fold; the most pronounced redn., about 40-fold, was obsd. for the B-type deletions occurring in the KB hybrids. Protein synthesis at 37.degree. is required for production of the B-type deletions at the high frequency. The redn. in the generation of deletions when the cells are grown at 42.degree. may be due to temp. sensitivity of a protein. DNA replication is probably not required for the event that culminates in a B-type deletion since, under certain conditions, deletion events occur in a thymidine auxotroph apparently in the absence of thymidine. The effects of thymidine starvation, however, are complicated and depend on whether a preceding period of amino acid starvation is allowed. The bacterial *recA* and *recB* functions are not required for the production of the B-type deletions.

L7 ANSWER 33 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1970:484864 CAPLUS
DOCUMENT NUMBER: 73:84864
TITLE: Effect of the "ribonucleic acid control" locus in *Escherichia coli* on T4 bacteriophage-specific ribonucleic acid synthesis
AUTHOR(S): Skold, Ola
CORPORATE SOURCE: Fac. Pharm., Univ. Uppsala, Stockholm, Swed.
SOURCE: J. Virol. (1970), 5(6), 718-25
CODEN: JOVIAM
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Amino acid control of RNA synthesis in bacteria is known to be governed genetically by the *rel* locus. We investigated whether the *rel* gene of the host would also exert its effect on the regulation of phage-specific RNA synthesis in T4 phage-infected *E. coli* cells. Since T-even phage infection completely shuts off host macromol. synthesis, phage RNA synthesis could be followed specifically by the cumulative incorporation of radioactivity from labeled precursors into RNA of infected cells. Labeled uracil accumulated in phage-specific RNA for 30-35 min after infection, a phenomenon which probably reflects an expansion of the labile phage-RNA pool. Amino acid starvation was effected by the use of auxotrophic bacterial strains or thienylalanine. The latter substance is an amino acid analog which induces a chem. auxotrophy by inhibiting the biosynthesis of phenylalanine, tyrosine, and tryptophan. Phage RNA synthesis was strictly dependent on the presence of amino acids, whereas phage DNA synthesis was not. By the use of several pairs of bacterial strains which were isogenic except for the *rel* gene, it was demonstrated that amino acid dependence was related to the allelic state of this gene. If the *rel* gene was mutated, amino acid starvation did not restrict phage RNA synthesis.

L7 ANSWER 34 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1970:75976 CAPLUS
DOCUMENT NUMBER: 72:75976
TITLE: Genetic analysis in *Bacillus pumilus* by [phage] PBS1-mediated transduction
AUTHOR(S): Lovett, Paul S.; Young, Frank E.
CORPORATE SOURCE: Dep. of Microbiol., Scripps Clin. and Res. Found., La Jolla, Calif., USA
SOURCE: J. Bacteriol. (1970), 101(2), 603-8
CODEN: JOBAAY

DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Bacteriophage PBS1 mediates generalized transduction in *B. pumilus* NRRL B-3275 (BpB1). Transduction frequencies for single auxotrophic markers are of the order of 10^{-4} transductants per plaque-forming unit in crude phage lysates. The characteristics of PBS1 propagated on BpB1 and the properties of the system of transduction are similar to those reported for PBS1 propagated on *B. subtilis*. By transduction, 8 amino acid auxotrophic markers in BpB1 have been oriented into 2 linkage groups. One group contains the auxotrophic markers arginine A, leucine, and phenylalanine, and the other group contains the markers lysine, serine, tryptophan, isoleucine-valine, and isoleucine. The nature and relative order of the markers within each linkage group suggest that the arrangement of genes in these areas of the chromosome of BpB1 is similar to the arrangement of phenotypically comparable genes in 2 linkage groups (defined by PBS1 transduction) in *B. subtilis*. However, transduction of any of the above cited markers in BpB1 to prototrophy with PBS1 propagated on *B. subtilis* 168 could not be demonstrated. In addn. to BpB1, 7 other strains of *B. pumilus* can be infected with PBS1. Transduction has been demonstrated in 3 of these strains.

L7 ANSWER 35 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1970:19378 CAPLUS
 DOCUMENT NUMBER: 72:19378
 TITLE: Temperature-sensitive induction of bacteriophage in *Bacillus subtilis* 168
 AUTHOR(S): Siegel, Eli C.; Marmur, Julius
 CORPORATE SOURCE: Albert Einstein Coll. of Med., Bronx, N. Y., USA
 SOURCE: J. Virol. (1969), 4(5), 610-18
 CODEN: JOVIAM
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB In a temp.-sensitive mutant of *B. subtilis* 168, induction of the defective phage PBSX occurred at 48.degree.. Cell lysis began after 90 min of growth at 48.degree., and cell viability began to decrease after 10 to 30 min. The loss in viability at the nonpermissive temp. was prevented by azide or cyanide. DNA, ribonucleic acid, and protein synthesis were not inhibited at 48.degree.. Temp. induction of the temperate phage SPO2 also occurred in this mutant. The temp.-sensitive mutation, designated tsi-23, was linked by transduction to purB6 and pig, the order being purB6 pig tsi-23. Mutation tsi-23 was transformable to wild type by *B. subtilis* 168 DNA but not by DNA from the closely related strains W23 or S31. DNA from the latter 2 strains transformed auxotrophic markers of strain 168 at frequencies close to those found with 168 donor DNA. Upon temp. induction, cellular DNA was broken to a size of 22 S, characteristic of DNA in PBSX particles. The DNA isolated from temp.-induced PBSX did not give an increased Ade+/Met+ transformant ratio relative to cellular DNA nor contain preferential break points as detected by transformation of four closely linked markers.

L7 ANSWER 36 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1969:55208 CAPLUS
 DOCUMENT NUMBER: 70:55208
 TITLE: Induction of a defective phage and DNA methylation in *Escherichia coli* 15T-
 AUTHOR(S): Medoff, Gerald; Swartz, Morton N.
 CORPORATE SOURCE: Harvard Med. Sch., Boston, Mass., USA
 SOURCE: J. Gen. Virol. (1969), 4(Pt. 1), 15-27
 CODEN: JGVIA Y
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Growth of the thymine auxotroph, *E. coli* 15T-, in a thymine 2.5 .mu.g./ml.-contg. medium with such antibacterial agents as 5-aminouracil (10-3M), mitomycin C (0.25 .mu.g./ml.), nalidixic acid (40 .mu.g./ml.), or hydroxyurea (8 mg./ml.) increased the 6-methyladenine content of extractable DNA and concomitantly increased DNA methylase activity. Thymine deprivation for 45 min. similarly affected these cells, but other antibacterial agents, such as penicillin G, phenethyl alc., and novobiocin did not. Assocd. with increased 6-methyladenine content and DNA methylase activity was the induction of defective bacteriophage. Similar effects were seen in wild-type *E. coli* 15. However, neither phage particles nor changes in DNA methylase or 6-methyladenine content were seen in *Escherichia coli* 15T- (a thymine auxotroph derived from 15T- with resistance to mitomycin C) under conditions leading to such effects in 15T-. This suggested that the rapid killing seen when logarithmically growing 15T- cells are transferred to a medium free of thymine is secondary to defective bacteriophage induction and that the increases in 6-methyladenine content and DNA methylase that occur in 15T- are phage-induced changes.

L7 ANSWER 37 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1968:433719 CAPLUS
 DOCUMENT NUMBER: 69:33719
 TITLE: Ribonucleic acid synthesis in T2-infected
 Escherichia coli during stringent control
 AUTHOR(S): Sharp, Karen; Green, Melvin H.
 CORPORATE SOURCE: Univ. of California, La Jolla, Calif., USA
 SOURCE: J. Bacteriol. (1968), 96(1), 111-16
 CODEN: JOBAAY
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The leucine auxotroph E. coli 2961 exhibited stringent control of net RNA synthesis during amino acid starvation. After leucine was exhausted from the medium, the rate of uracil incorporation into RNA rapidly decreased to 2-4% of the prestarvation value. Infection of the starved cells with T2 phage stimulated uracil incorporation to a level equiv. to that of unstarved, T2-infected cells. The RNA synthesized during leucine starvation of the T2-infected cells consisted of T2 and E. coli messenger RNA, but stable ribosomal RNA (23S and 16S) did not appear to be synthesized. One or more T2-specific proteins are probably required to shut off host messenger RNA synthesis. Transcription of E. coli and T2 DNA is not necessarily couple to the translation of messenger RNA during stringent control of net RNA synthesis. 25 references.

L7 ANSWER 38 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1967:479946 CAPLUS
 DOCUMENT NUMBER: 67:79946
 TITLE: Regulation mechanism of enzyme synthesis in tryptophan biosynthetic pathway of Escherichia coli. I. Biosynthesis of tryptophan synthetase controlled by phage-carrying genes
 AUTHOR(S): Kida, Setsuko; Matsushiro, Aizo; Ito, Junetsu; Sato, Koki
 CORPORATE SOURCE: Osaka Univ., Osaka, Japan
 SOURCE: Hakko Kogaku Zasshi (1965), 43(4), 233-9
 CODEN: HKZAA2
 DOCUMENT TYPE: Journal
 LANGUAGE: Japanese

AB The biosynthesis of tryptophan synthetase by E. coli, after infection with .vphi.80 HFT lysate was studied. The transducing particles .vphi.80 dt present in the HFT lysate incorporate various lengths of the tryptophan operon. The first type of transducing particle, .vphi.80 dt0, is able to transduce all tryptophan-requiring mutants, implying that all structural genes of the tryptophan operon are incorporated into the phage genome. When the HFT lysate contg. this kind of transducing particle infects the A2-tryptophan auxotroph, the synthesis of tryptophan synthetase is repressed by the presence of tryptophan. The second type of transducing particles, .vphi.80 dt1, and .vphi.80 dt2, cannot transduce anthranilic acid mutants. These transducing particles do not possess the gene involved in the synthesis of anthranilic acid synthetase. When the HFT lysate contg. the second type of transducing particle infects the A2 tryptophan auxotroph, the synthesis of tryptophan synthetase is not repressed even in the presence of tryptophan in the medium. These observations suggest that a gene concerned with the regulation of tryptophan synthetase is assocd. with the anthranilic acid synthetase locus or closely linked to this locus: 16 references.

L7 ANSWER 39 OF 48 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1962:55860 CAPLUS
 DOCUMENT NUMBER: 56:55860
 ORIGINAL REFERENCE NO.: 56:10673b-f
 TITLE: Properties of an arginine auxotroph of Salmonella typhimurium
 AUTHOR(S): Clarke, C. H.
 CORPORATE SOURCE: Inst. Animal Genetics, Edinburgh, UK
 SOURCE: Experientia (1961), 17, 499-501
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The arginine (arg) auxotroph G-12 was isolated in an expt. in which stationary-phase cells from an aerated nutrient broth culture of S. typhimurium strain LT-22 were treated with MnCl2, grown in broth, screened with penicillin, and then plated on mineral medium A (Davis and Mingioli, CA 44, 9512c) supplemented with a limiting concn. of L-arginine (1 .gamma./ml.). The auxotroph G-12 was found among the minute colonies which developed alongside the larger prototrophic colonies. Auxotroph G-12 responded only to L-arginine and showed no growth response to ornithine or citrulline. It was not inhibited by L-lysine and grew well on complex media. G-12 cells survived some 50 times better than prototrophic cells on exposure to penicillin screening. No reversions to arginine independence were found among cells from 24 independent broth cultures. When cells from a broth culture of G-12 were irradiated with ultraviolet light to 11.3%

survival, then incubated on minimal A plates supplemented with 1% liquid nutrient, arg⁺ reversions arose at the rate of 0.3/106 survivors. MnCl₂ treatment to 63% survival gave 0.05 arg⁺ reversions/106 survivors. Transductional tests were carried out with bacteriophage PLT-22 grown on prototrophic bacteria. G-12 was infected at a multiplicity of 20 and plated on limitedly enriched minimal A. Transductions to arg⁺ occurred at a rate of 1/107 phage particles. The effect of chem. mutagens was ascertained (Iyer and Szybalski, CA 52, 9285g). Neg. results were obtained with diepoxybutane, caffeine, 5-bromouracil + aminopterin, and H₂O₂ and CH₂O. .beta.Propiolactone gave a few reversions, and numerous reversions were obtained after treatment with MeSO₃Et. Neg. results were obtained in all attempted hybridizations of G-12 with other enteric bacteria.

L7 ANSWER 40 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1997:451479 BIOSIS

DOCUMENT NUMBER: PREV199799750682

TITLE: Generalized gene transfer by virus-like particles from marine bacteria.

AUTHOR(S): Chiura, Hiroshi X.

CORPORATE SOURCE: Dep. Biol., Div. Natural Sci., International Christian Univ., 3-10-2 Osawa, Mitaka, Tokyo 181 Japan

SOURCE: Aquatic Microbial Ecology, (1997) Vol. 13, No. 1, pp. 75-83.

ISSN: 0948-3055.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Spontaneous VLP (virus-like particle) production and VLP-mediated gene transfer into *Escherichia coli* AB1157 as recipient was demonstrated. Five marine isolates (Alc 096, Alc 233, Alc 252, *Agrobacterium kieliiense* and *Flavobacterium* sp. 11604) were investigated for their potential to produce VLP as well as for the gene transfer capability of these VLPs to the *E. coli* recipient. These strains are classified as ubiquinone-10-possessing marine bacteria (Q10MB) in the 16S-rRNA Superfamily IV. VLPs were obtained from 100 h cultured broth of all strains examined. VLP-host ratio after 100 h growth culture was: Alc 233, 1.54; Alc 252, 1.26; Alc 096, 1.06; *Flavobacterium* sp. 11604, 0.69; and *A. kieliiense*, 0.06. These ratios were smaller than those found in the marine environment. However, the spontaneously produced VLP number can be considered as high because the reported numbers are relatively low from coliphage lambda (0.005) and phage Mu (apprx 0.0001). VLP-mediated gene transfer was examined using an auxotrophic mutant of *E. coli* (AB1157) with 4 amino acid deficiencies (leu, pro, his, arg) as recipient at multiplicity of infection (MOI) of 0.1. Through this treatment, VLPs showed lethal effect on the recipient. The survival rate of control was: Alc 096, 7%; Alc 252, 8%; *A. kieliiense*, 17%; *Flavobacterium* sp. 11604, 31%; and Alc 233, 40%. At the same time, all the purified VLPs derived from these 5 strains successfully transferred genes to rescue genetic defects of the recipient. Overall average efficiency of VLP-mediated gene transfer at MOI of 0.1 was estimated to be between 2.62 times 10⁻³ and 3.58 times 10⁻⁵ per VLP particle. Loci of employed genetic markers were dispersed on the *E. coli* chromosome with mutual distance of 121, 1154, 1397 and 364 kb between them. Since VLPs from different sources showed similar gene transfer efficiency in respect to the genetic marker rescued, it is suggested that VLPs from Q10MB transferred genes as generalized transduction. These results indicate that the VLPs produced by certain marine bacteria may be an important element for both non-specific generalized horizontal gene transfer towards a broad range of bacterial hosts and population control in the marine environment.

L7 ANSWER 41 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1997:386183 BIOSIS

DOCUMENT NUMBER: PREV199799685386

TITLE: Bioincorporation of telluromethionine into proteins: A promising new approach for X-ray structure analysis of proteins.

AUTHOR(S): Budisa, Nediljko (1); Karnbrock, Wilhelm; Steinbacher, Stefan; Humm, Andreas; Prade, Lars; Neufeld, Torsten; Moroder, Luis; Huber, Robert

CORPORATE SOURCE: (1) Max-Planck Inst. Biochem., Martinsreid Germany

SOURCE: Journal of Molecular Biology, (1997) Vol. 270, No. 4, pp. 616-623.

ISSN: 0022-2836.

DOCUMENT TYPE: Article

LANGUAGE: English

AB A simple and efficient method for the specific and quantitative replacement of the naturally occurring amino acid methionine by its isosteric analogue telluromethionine in the expression of recombinant proteins has been developed. The method requires a controllable and competitive expression system like the bacteriophage T7 polymerase/promoter in a methionine-auxotrophic host. Using methionine-auxotrophic *Escherichia coli* strains, incorporation

of telluromethionine at high yields has been achieved for human recombinant annexin V, human mitochondrial transamidase, Arabidopsis glutathione-S-transferase and the N-terminal domain of Salmonella tailspike adhesion protein as confirmed by amino acid, mass-spectrometric and X-ray analyses. Expressed and purified telluromethionine-proteins and native proteins were found to crystallize isomorphously. In terms of efficient bio-expression, isomorphism of crystals and relative abundance of methionine residues, the production of telluromethionine-proteins as heavy-atom derivatives offers a valid and general approach in X-ray analysis by the method of multiple isomorphous replacement.

L7 ANSWER 42 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1995:351339 BIOSIS

DOCUMENT NUMBER: PREV199598365639

TITLE: High-level expression and isotopic labeling of *Lactobacillus casei* dihydrofolate reductase for nuclear magnetic resonance spectroscopy.

AUTHOR(S): Badii, Ramin (1); Basran, Jaswir; Casarotto, Marco G.; Roberts, Gordon C. K.

CORPORATE SOURCE: (1) Biol. NMR Cent., Univ. Leicester, P.O. Box 138, Med. Sci. Build., University Road, Leicester LE1 9HN UK

SOURCE: Protein Expression and Purification, (1995) Vol. 6, No. 3, pp. 237-243.
ISSN: 1046-5928.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Two efficient systems have been used for high-level expression of *Lactobacillus casei* dihydrofolate reductase in *Escherichia coli*, including the production of protein generally and specifically labeled with ¹³C and ¹⁵N. A system based on T7 RNA polymerase led to the production of dihydrofolate reductase at a level of 37% of the total soluble protein of the host strain: 50 mg of pure enzyme was obtained from a 1 liter of culture (or 14 mg/g wet weight of cells). In this system, a small amount of the enzyme (less than 5%) was identified as a catalytically active 21-kDa fusion protein. Introduction of a second in-frame (ochre) stop codon did not eliminate the production of this fusion protein. The same expression system was also used to prepare dihydrofolate reductase generally labeled with ¹⁵N and to prepare single and double mutants of the enzyme. In order to have an expression system which can be used with a range of auxotrophic strains of *E. coli*, a system based on the tac promoter was used. This led to the production of dihydrofolate reductase at a level of 29% of total soluble protein; a yield of 40 mg enzyme per liter of culture (or 11 mg/g wet weight of cells). This system was successfully used to produce mutants of the enzyme as well as the enzyme selectively labeled with (gamma-¹³C)aspartic acid.

L7 ANSWER 43 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1993:499193 BIOSIS

DOCUMENT NUMBER: PREV199396123200

TITLE: Bacteriophage lambda as a delivery vector for Tn10-derived transposons in *Xenorhabdus bovienii*.

AUTHOR(S): Francis, Matthew S.; Parker, Angela F.; Morona, Renato; Thomas, Connor J.

CORPORATE SOURCE: Dep. Microbiol. Immunol., Univ. Adelaide, G.P.O. Box 498, Adelaide, South Australia, Australia 5001

SOURCE: Applied and Environmental Microbiology, (1993) Vol. 59, No. 9, pp. 3050-3055.
ISSN: 0099-2240.

DOCUMENT TYPE: Article

LANGUAGE: English

AB *Xenorhabdus bovienii* wild-type strains lack a functional receptor protein (LamB) in the outer membrane and as a result are unable to adsorb coliphage lambda (lambda). Introduction of plasmids encoding lamB into *X. bovienii* T228 results in constitutive expression of LamB in the outer membrane of this organism. LamB-expressing strains of *X. bovienii* adsorb lambda bacteriophage particles and can be used as hosts for lambda::Tn constructs. A Tn10-derived transposon, element 9 (J. C. Way, D. Davis, D. Morisato, D. E. Roberts, and N. Kleckner, Gene 32: 369-379, 1984) was used to construct a variety of insertion mutants of *X. bovienii*. Mutants that had altered expression of protease, lipase, DNase, dye-binding capability, and hemolytic activity, in addition to a series of auxotrophic mutants, were isolated.

L7 ANSWER 44 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1986:142086 BIOSIS

DOCUMENT NUMBER: BA81:52502

TITLE: BACTERIOPHAGE SPO-2-MEDIATED PLASMID TRANSDUCTION IN TRANSPOSITIONAL MUTAGENESIS WITHIN THE GENUS *BACILLUS*.

AUTHOR(S): KOPEC L K; YASBIN R E; MARRERO R

CORPORATE SOURCE: DEP. MICROBIOLOGY, UNIV. ROCHESTER MED. CENTER, ROCHESTER, NEW YORK 14642.

SOURCE: J BACTERIOL, (1985) 164 (3), 1283-1287.
 CODEN: JOBAAY. ISSN: 0021-9193.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English

AB A single copy of the *Streptococcus faecalis* transposon Tn917, located in the *Bacillus subtilis* chromosome, was able to transpose onto the SPO2 cos plasmid pPL1017, which codes for chloramphenicol resistance and contains the bacteriophage .PHI.105 immunity region. Selection for pPL1017::Tn917 chimeras was performed by SPO2-mediated plasmid transduction of transposon-borne resistance to macrolide-lincosamide-streptogramin B antibiotics (MLSr). The transposition of Tn917 onto plasmid pPL1017 occurred with a frequency of 10^{-5} and was dependent on the presence of a subinhibitory dose of erythromycin. Twelve chimeras were subjected to genetic and physical analyses. Two Cams transductants harbored plasmids whose chloramphenicol acetyltransferase genes had been insertionally inactivated by Tn917. Several transpositions in the vicinity of the .PHI.105 immunity region were detected. However, all of the 300 MLSr, Camr transductants screened were immune to .PHI.105 infectious activity. One pPL1017::Tn917 chimera, pLK200, was transferred by SPO2 plasmid transduction into the *Bacillus amyloliquefaciens* prototrophic strain DSM7. Plasmid pLK200 was effective in the mutagenesis of the DSM7 chromosome and yielded auxotrophs at a frequency of 0.5 to 5.3%. Generation of auxotrophs was also dependent on the presence of a subinhibitory dose of erythromycin. Forty-four auxotrophs representing at least nine amino acid requirements were recovered.

L7 ANSWER 45 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1984:354852 BIOSIS
 DOCUMENT NUMBER: BA78:91332
 TITLE: INTER VARIANT TRANSDUCTION IN BACILLUS-THURINGIENSIS.
 AUTHOR(S): POGOSBEKOVA M R; AZIZBEKYAN R R
 CORPORATE SOURCE: ALL-UNION RES. INST. GENET. SEL. IND. MICROORG., MOSCOW, USSR.
 SOURCE: GENETIKA, (1984) 20 (1), 187-189.
 CODEN: GNKAA5. ISSN: 0016-6758.
 FILE SEGMENT: BA; OLD
 LANGUAGE: Russian

AB Intervariant transduction was studied in strains of *B. thuringiensis* ssp. *dendrolimus* and *B. thuringiensis* ssp. *galleriae* with bacteriophage Tg13. This bacteriophage has a broad host range and is of a large DNA molecular mass (40.3 megadaltons). Transducing properties of this bacteriophage were studied in *B. thuringiensis* ssp. *galleriae* using auxotrophic mutants requiring purine and pyrimidine bases or tryptophan as recipients. The frequency of transduction was 10^{-5} - 10^{-6} . To prove the possibility of intervariant transduction, 2 series of experiments were conducted. First, bacteriophage Tg13 was propagated on the prototrophic strain 49-18 (ssp. *dendrolimus*), 48 gua (ssp. *galleriae*) being used as a recipient. In the 2nd case, the prototrophic strain 69-6 (ssp. *galleriae*) served as a donor and 49-18-1 trp (ssp. *dendrolimus*) as a recipient. The frequency of heterologous transduction was 10^{-7} . The positive results obtained in cross-transduction experiments may be explained by common metabolic pathways of purine and pyrimidine bases and amino acids in strains of different subspecies.

L7 ANSWER 46 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1977:186027 BIOSIS
 DOCUMENT NUMBER: BA64:8391
 TITLE: A NOVEL PLEIOTROPIC MUTATION IN ESCHERICHIA-COLI K-12 WHICH AFFECTS TRANSDUCTION TRANSFORMATION AND RATES OF MUTATION.
 AUTHOR(S): STACEY K A; OLIVER P
 SOURCE: J GEN MICROBIOL, (1977) 98 (2), 569-578.
 CODEN: JGMIAN. ISSN: 0022-1287.
 FILE SEGMENT: BA; OLD
 LANGUAGE: Unavailable

AB A mutant strain of *E. coli* K12, R2721, differs from its parent strain, S491, in 4 associated phenotypic characters as a result of a single mutation. This strain did not give recombinants with DNA transduced by bacteriophage P1 or bacteriophage Mu, nor transformants after exposure to R factor DNA; lysates of bacteriophage P1 grown on this strain did not appear to contain any transducing particles when tested on normal recipients. The reversion rates, both spontaneous and UV-induced, for 2 auxotrophic markers were reduced. The frequency of revertants was at least 2 orders of magnitude lower in cultures of R2721 than in cultures of S491. Many of the rare revertants for one or other of the auxotrophic markers regained normal reversion frequencies for the other marker and for the capacity to be transduced. In all other respects, recombination in R2721 appeared normal, the frequency of chromosomal mobilization by an F' factor was unaffected and normal yields of recombinants were obtained from matings with Hfr strains. The only circumstance in which transduction of R2721 was observed was when the capacity to ferment galactose was selected and P1

was grown on a strain carrying .lambda.dgal when, presumably, integration was effected by the phage-coded gene products. The mutation was located on the E. coli chromosome map between tonA and pro and was given the symbol tdi (transduction inhibition). Double mutants, (tdi recA) and (tdi recB), were isolated and show no unexpected properties.

L7 ANSWER 47 OF 48 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 92289006 EMBASE

DOCUMENT NUMBER: 1992289006

TITLE: Identification of the Escherichia coli murI gene, which is required for the biosynthesis of D-glutamic acid, a specific component of bacterial peptidoglycan.

AUTHOR: Doublet P.; Van Heijenoort J.; Mengin-Lecreulx D.

CORPORATE SOURCE: URA 1131 CNRS, Universite Paris-Sud, Batiment 432, 91405 Orsay, France

SOURCE: Journal of Bacteriology, (1992) 174/18 (5772-5779).

ISSN: 0021-9193 CODEN: JOBAAAY

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The murI gene of Escherichia coli, whose inactivation results in the inability to form colonies in the absence of D-glutamic acid, was identified in the 90-min region of the chromosome. The complementation of an auxotrophic E. coli B/r strain by various DNA sources allowed us to clone a 2.5-kbp EcoRI chromosomal fragment carrying the murI gene into multicopy plasmids. The murI gene corresponds to a previously sequenced open reading frame, ORF1 (J. Brosius, T. J. Dull, D. D. Sleeter, and H. F. Noller. J. Bacteriol. 148:107-127, 1987), located between the btuB gene, encoding the vitamin B12 outer membrane receptor protein, and the rrnB operon, which contains the genes for 16S, 23S, and 5S rRNAs. The murI gene product is predicted to be a protein of 289 amino acids with a molecular weight of 31,500. Attempts to identify its enzymatic activity were unsuccessful. Cells altered in the murI gene accumulate UDP-N-acetylmuramyl-L-alanine to a high level when depleted of D-glutamic acid. Pools of precursors located downstream in the pathway are consequently depleted, and cell lysis finally occurs when the peptidoglycan content is 25% lower than that of normally growing cells.

L7 ANSWER 48 OF 48 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 84006651 EMBASE

DOCUMENT NUMBER: 1984006651

TITLE: Localized hydroxylamine mutagenesis, and cotransduction of threonine and lysine genes, in Streptomyces venezuelae.

AUTHOR: Stuttard C.

CORPORATE SOURCE: Microbiol. Dep., Dalhousie Univ., Halifax, NS B3H 4H7, Canada

SOURCE: Journal of Bacteriology, (1983) 155/3 (1219-1223).

CODEN: JOBAAAY

COUNTRY: United States

DOCUMENT TYPE: Journal

FILE SEGMENT: 004 Microbiology

022 Human Genetics

LANGUAGE: English

AB A lysate of the generalized transducing phage SV1, grown on the prototrophic type strain 10712 of Streptomyces venezuelae, was mutagenized with hydroxylamine and used to transduce a lysineless auxotroph to lysine independence on supplemented minimal agar. A complex threonine mutant, strain VS95, was isolated from among the transductants and was shown to be carrying at least two different thr mutations. These were about 50% cotransducible with alleles of four independently isolated lysA mutations, as were two other independently isolated threonine mutations, thr-1 and hom-5. The location of thr genes close to lysA occurs in at least three other streptomycetes, but apparently not in Streptomyces coelicolor A3(2), in which the lysA and thr loci are at diametrically opposite locations on the linkage map. This first observation of cotransduction between loci governing the biosynthesis of different amino acids in the genus Streptomyces demonstrates the feasibility of fine-structure genetic analysis by transduction in these antibiotic-producing bacteria.

L8 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:31439 CAPLUS
TITLE: Method of preventing bacteriophage infection
of bacterial cultures
INVENTOR(S): Nilsson, Dan; Janzen, Thomas
PATENT ASSIGNEE(S): Chr. Hansen A/s, Den.
SOURCE: PCT Int. Appl.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000001799	A2	20000113	WO 1999-DK382	19990702
WO 2000001799	A3	20010322		
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9947691	A1	20000124	AU 1999-47691	19990702
BR 9912502	A	20010502	BR 1999-12502	19990702
EP 1102837	A2	20010530	EP 1999-931021	19990702
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
NO 2000006428	A	20010214	NO 2000-6428	20001215
PRIORITY APPLN. INFO.:			DK 1998-878	A 19980703
			US 1998-91735	P 19980706
			WO 1999-DK382	W 19990702

AB Method of preventing bacteriophage infection of bacterial cultures comprising modified strains, wherein the cultures are completely resistant to bacteriophage attack and have retained their capability of being metabolically active. The method is useful in the manufacturing of food products, feed products or useful metabolite products.

L8 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:90258 CAPLUS
DOCUMENT NUMBER: 130:150861
TITLE: Nonsense suppressor mutants of lactic acid bacteria and their use in the containment of plasmids in lactic acid bacteria
INVENTOR(S): Dickely, Francedillaioise; Johansen, Eric; Nilsson, Dan; Hansen, Egon Bech
PATENT ASSIGNEE(S): Chr. Hansen A/S, Den.
SOURCE: U.S., 44 pp., Cont.-in-part of U.S. 5,691,185.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5866385	A	19990202	US 1995-535092	19951205
US 5691185	A	19971125	US 1994-242098	19940513
WO 9510621	A1	19950420	WO 1994-DK376	19941007
W:	AM, AT, AT, AU, BB, BG, BR, BY, CA, CN, CZ, CZ, DE, DE, DK, DK, EE, FI, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SI, SK, SK, TJ, TT, UA, US, UZ, VN			
RW:	KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
PRIORITY APPLN. INFO.:			US 1993-133390	19931008
			US 1994-242098	19940513
			WO 1994-DK376	19941007

AB Methods of isolating lactic acid bacteria carrying nonsense suppressor mutations that can be used to contain plasmids are described. In particular, the mutants are used to stabilize plasmids in mixed cultures used in food processing. The process involves creating a nonsense mutation in an essential gene, such as an antibiotic resistance marker, of a plasmid and then screening mutagenized host cells for stabilization of the plasmid. The development of suppressor systems using tRNA^{Ser} and tRNA^{Gln} amber suppressors to suppress amber mutations in plasmids and bacteriophages in *Lactococcus lactis* is described. A pepN gene for lysine

aminopeptidase on a plasmid stabilized by an amber mutation showed higher levels of expression and a 4-5-fold increase in aminopeptidase activity in the host cell. The plasmid was stable in cultures grown in a milk/casamino acids medium.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:761700 CAPLUS
DOCUMENT NUMBER: 128:59318
TITLE: Nonsense suppressors of lactic acid bacterial suppressor and their use as selective markers and in containment of lactic acid bacteria
INVENTOR(S): Dickely, Francedillaioise; Johansen, Eric; Nilsson, Dan; Hansen, Egon Bech; Stroslassedman, Per
PATENT ASSIGNEE(S): Chr. Hansen A/S, Den.
SOURCE: U.S., 40 pp. Cont.-in-part of U.S. Ser. No. 133,390, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5691185	A	19971125	US 1994-242098	19940513
WO 9510621	A1	19950420	WO 1994-DK376	19941007
W: AM, AT, AU, BB, BG, BR, BY, CA, CN, CZ, DE, DK, EE, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SI, SK, TJ, TT, UA, US, UZ, VN				
RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2160166	AA	19950420	CA 1994-2160166	19941007
AU 9478529	A1	19950504	AU 1994-78529	19941007
AU 684556	B2	19971218		
EP 722503	A1	19960724	EP 1994-929490	19941007
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
US 5866385	A	19990202	US 1995-535092	19951205
PRIORITY APPLN. INFO.:				
			US 1993-133390	19931008
			US 1994-242098	19940513
			WO 1994-DK376	19941007

AB A method of screening for nonsense suppressors in lactic acid bacteria for use in the containment of a suppressor-dependent replicon is described. The method involves using a plasmid-borne nonsense mutation, e.g. in antibiotic resistance gene. The plasmid is introduced into a mutated population of lactic acid bacteria and cells carrying a nonsense suppressor that can support replication can be selected for using the antibiotic resistance as a selectable marker. A replicon carrying the same nonsense mutation can only survive in a host carrying this particular suppressor making the suppressor gene an effective way of contg. the replicon in a specific host. Such containment systems are intended for use in food processing. An amber suppressing glutamine tRNA was prepd. and characterized. The development of food grade plasmids using this system is also described.

L8 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:652525 CAPLUS
DOCUMENT NUMBER: 123:29474
TITLE: Lactic acid bacterial suppressor mutants and their use as selective markers and means of containment in lactic acid bacteria
INVENTOR(S): Dickely, Francoise; Johansen, Eric; Nilsson, Dan; Hansen, Egon Bech
PATENT ASSIGNEE(S): Chr. Hansen's Laboratorium Danmark, Den.
SOURCE: PCT Int. Appl., 117 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9510621	A1	19950420	WO 1994-DK376	19941007
W: AM, AT, AU, BB, BG, BR, BY, CA, CN, CZ, DE, DK, EE, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SI, SK, TJ, TT, UA, US, UZ, VN				
RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN,				

TD, TG

US 5691185	A	19971125	US 1994-242098	19940513
AU 9478529	A1	19950504	AU 1994-78529	19941007
AU 684556	B2	19971218		
EP 722503	A1	19960724	EP 1994-929490	19941007
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
US 5866385	A	19990202	US 1995-535092	19951205
PRIORITY APPLN. INFO.:			US 1993-133390	19931008
			US 1994-242098	19940513
			WO 1994-DK376	19941007

AB Disclosed are mutants of lactic acid bacteria or plasmids capable of replicating in lactic acid bacteria, comprising nonsense mutation suppressor-encoding genes. The method comprises (1) mutagenizing a selectable marker-contg. replicon capable of replicating in lactic acid bacteria and selecting a replicon mutant contg. a nonsense mutation in the gene encoding the marker, (2) mutagenizing lactic acid bacteria that do not encode a suppressor, (3) introducing the replicon mutant of (1) into the mutagenized lactic acid bacteria, and (4) selecting suppressor-encoding lactic acid bacteria in which the marker is expressed. Prepn. of shuttle plasmid pFDi10 carrying mutations in 2 antibiotic resistance genes, selection of nonsense-suppressing strains of *Lactococcus lactis* using pFDi10,. The use of such suppressor genes for confining a replicon to a specific lactic acid bacterium or to a lactic acid bacterium growing in a particular environment and for controlling the no. of lactic acid bacterial cells in a particular environment is also described.

L8 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:482884 CAPLUS
 DOCUMENT NUMBER: 123:75905
 TITLE: Isolation of *Lactococcus lactis* nonsense suppressors and construction of a food-grade cloning vector
 AUTHOR(S): Dickely, Françoise; Nilsson, Dan; Hansen, Egon Bech; Johansen, Eric
 CORPORATE SOURCE: Dep. Genet., Christian Hansen's Laboratorium Danmark A/S, Hørsholm, DK-2970, Den.
 SOURCE: Mol. Microbiol. (1995), 15(5), 839-47
 CODEN: MOMIEE; ISSN: 0950-382X
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Nonsense suppressor strains of *Lactococcus lactis* were isolated using plasmids contg. nonsense mutations or as revertants of a nonsense auxotrophic mutant. The nonsense suppressor gene was cloned from two suppressor strains and the DNA sequence detd. One suppressor is an ochre suppressor with an altered tRNA^{Arg} and the other an amber suppressor with an altered tRNA^{Ser}. The nonsense suppressors allowed isolation of nonsense mutants of a lytic bacteriophage and suppressible auxotrophic mutants of *L. lactis* MG1363. A food-grade cloning vector based totally on DNA from *Lactococcus* and a synthetic poly-linker with 11 unique restriction sites was constructed using the ochre suppressor as a selectable marker. Selection, following electroporation of a suppressible purine auxotroph, can be done on purine-free medium. The pepN gene from *L. lactis* Wg2 was sub-cloned resulting in a good-grade plasmid giving a four- to fivefold increase in lysine aminopeptidase activity.

L9 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:31439 CAPLUS
 TITLE: Method of preventing bacteriophage infection
 of bacterial cultures
 INVENTOR(S): Nilsson, Dan; Janzen, Thomas
 PATENT ASSIGNEE(S): Chr. Hansen A/s, Den.
 SOURCE: PCT Int. Appl.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000001799	A2	20000113	WO 1999-DK382	19990702
WO 2000001799	A3	20010322		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG AU 9947691 A1 20000124 AU 1999-47691 19990702 BR 9912502 A 20010502 BR 1999-12502 19990702 EP 1102837 A2 20010530 EP 1999-931021 19990702 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO NO 2000006428 A 20010214 NO 2000-6428 20001215 PRIORITY APPLN. INFO.: DK 1998-878 A 19980703 US 1998-91735 P 19980706 WO 1999-DK382 W 19990702				

AB Method of preventing bacteriophage infection of bacterial cultures comprising modified strains, wherein the cultures are completely resistant to bacteriophage attack and have retained their capability of being metabolically active. The method is useful in the manufacturing of food products, feed products or useful metabolite products.

L9 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1992:528110 CAPLUS
 DOCUMENT NUMBER: 117:128110
 TITLE: Mechanism of plasmid-mediated bacteriophage resistance in lactococci
 AUTHOR(S): Geis, A.; Janzen, T.; Teuber, M.; Wirsching, F.
 CORPORATE SOURCE: Inst. Mikrobiol., Bundesanst. Milchwforsch., Kiel, D-2800, Germany
 SOURCE: FEMS Microbiol. Lett. (1992), 94(1-2), 7-13
 CODEN: FMLED7; ISSN: 0378-1097
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Plasmids pBul-B and p1149-3 from *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* Bul and *L. Lactis* ssp. *cremoris* 1149 mediate resistance against a variety of mostly isodiametrically headed bacteriophages. Plasmid pBul-8 was conjugally transferred into several plasmid-free, phage sensitive strains of both lactococcal subspecies rendering all transconjugants phage resistant. Infection with bacteriophages P008 and P416 labeled in vivo with the fluorescence dye 4'-6-diaminodino-2-phenylindole (DAPI) showed that adsorption and DNA injection took place with both sensitive and resistant strains even after extensive starvation of the host cells. In resistant cells carrying either of the plasmids, phage DNA was replicated >5 h post-infection. No transcription of the phage DNA, however, could be obsd.

From: Steadman, David (AU1652)
Sent: Tuesday, February 12, 2002 4:26 PM
To: STIC-ILL
Subject: Literature/ILL request

Art Unit: 1652
Office: 10D-04
Mailbox: 10C-01 M3
Case Serial #:09/720,096

Please provide the following references:

- 1) Nucleic Acids Res 1981 Dec 21;9(24):6975-84
Direction of bacteriophage lambda DNA replication in a thymine requiring Escherichia coli K-12 strain. Effect of thymidine concentration.
Valenzuela MS, Inman RB.
- 2) J Bacteriol 1984 Jan;157(1):126-9
Suppression by thymidine-requiring mutants of Escherichia coli K-12.
Herrington MB, Kohli A, Lapchak PH.
- 3) Dev Biol Stand 1995;85:531-4
Nonsense suppression in Lactococcus lactis: construction of a "food-grade" cloning vector.
Johansen E, Dickely F, Nilsson D, Hansen EB.

Thank you very much,
David J. Steadman
Art Unit 1652
CM1, 10D-04
308-3934

Direction of bacteriophage λ DNA replication in a thymine requiring *Escherichia coli* K-12 strain.
Effect of thymidine concentration

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WI 53706, USA

Received 12 October 1981

ABSTRACT

The direction of replication was established for the first round of bacteriophage λ DNA replication in thymine requiring *E. coli* K-12 cells exposed to different concentrations of thymidine. It was found that a dramatic decrease in the proportion of bidirectionally replicating molecules followed a decrease in the concentration of thymidine. Moreover, the rightward mode of replication appears to be exclusively favored in unidirectionally replicating molecules found at low concentrations of thymidine.

INTRODUCTION

During our studies on the first round of λ DNA replication, we have noticed that although replication commences at a unique region of the λ genome and appears to proceed in a bidirectional fashion, a low but significant proportion of the molecules replicate unidirectionally. Moreover, the rightward mode of replication appears to be favored in all λ variants studied so far (1,2). More recent studies have confirmed these observations and shown that the bias can be reversed in a λ variant in which the origin containing region has been inserted in opposite polarity (3). Since an understanding of the way unidirectionally replicating molecules are generated may throw some light on the initiation of DNA replication, we have investigated conditions that would alter the proportion of bidirectional and unidirectional molecules.

Very recently we have reported the isolation of novel DNA structures found among λ replicating intermediates, which showed a strong bias towards unidirectional replication (4). Since these molecules were found in infected cells that had been previously starved for thymidine, we have investigated the effect of thymidine concentration on the direction of the first round of λ DNA replication. The results presented below suggest that unidirectional replication is strongly favored at low concentrations of thymidine.

MATERIALS AND METHODS

(a) Bacterial and phage strains

E. coli K-12 CR34 (thy, leu, thr) and bacteriophage λ CI₆₇CI_{III}₆₈ were used in this study.

(b) Growth of labeled phages

Radioactive ³H-labeled phages were obtained by growing infected CR34 cells on agar plates in the presence of [methyl-³H]thymidine (Valenzuela, to be published elsewhere). Phages were purified by low and high speed centrifugation and resuspended in 0.01 M Tris, 0.01 M MgSO₄ in D₂O (pH 7.2).

(c) Growth of CR34 cells

CR34 cells were grown at 37°C in M9-maltose medium supplemented with 1% casamino acids and 5 µg/ml thymidine. At OD₅₉₀ = 0.2, cells were pelleted and washed twice in 0.02 M MgSO₄, 0.02 M Tris, pH 7.4, and resuspended into 2 ml of the same medium. Aliquots were poured into 5 ml M9-maltose medium plus casamino acids containing either 0, 0.2, 0.5 or 5 µg/ml thymidine. Cultures were incubated at 37°C and cell viability was checked at various times after transferring to new medium.

(d) Isolation of intracellular λ DNA

CR34 cells were grown at 37° in 20 ml D₂O-maltose medium (5) supplemented with yeast extract (.02%), leucine (10 µg/ml), threonine (10 µg/ml) and thymidine (5 µg/ml). At OD₅₉₀ = 0.60, cells were pelleted, washed twice with 10 ml of 0.01 M Tris, 0.01 M MgSO₄ in D₂O and resuspended in 2 ml of the same solution. After incubation at 37° for 1 hr, cells were infected with ³H-labeled λ phage at moi = 6 and adsorption allowed for 15 min at 37°. The cell suspension was then poured into warmed 20 ml D₂O-glucose medium supplemented with leucine and threonine each at 10 µg/ml and thymidine at either 5, 0.5, 0.2 or 0 µg/ml. After incubating the cells for 5-7 min at 37°, replication was stopped, cells lysed and λ DNA isolated according to a methodology described previously (6).

(e) Electron microscopy and computation of electron microscope data

λ DNA replicative intermediates were photographed at random and the position of branch points established with respect to the denaturation map by the method described by Valenzuela and Inman (6).

(f) Classification of directionality of replication

The method described by Schnös *et al.* (3) was followed.

and bacteriophage λ CII₆₇CIII₆₈ were

obtained by growing infected CR34
methyl-³H]thymidine (Valenzuela,
purified by low and high speed
Tris, 0.01 M MgSO₄ in D₂O (pH 7.2).

Maltose medium supplemented with
At OD₅₉₀ = 0.2, cells were
0.02 M Tris, pH 7.4, and
Aliquots were poured into 5 ml
containing either 0, 0.2, 0.5 or
at 37°C and cell viability was
to new medium.

D₂O-maltose medium (5) supplemented
1), threonine (10 µg/ml) and
s were pelleted, washed twice
D₂O and resuspended in 2 ml of
for 1 hr, cells were infected
uption allowed for 15 min at
into warmed 20 ml D₂O-glucose
ine each at 10 µg/ml and thymidine
ubating the cells for 5-7 min
and λ DNA isolated according

electron microscope data
photographed at random and the
respect to the denaturation map
ian (6).

cation
) was followed.

RESULTS

(a) Thymidine concentration, cell viability and λ infectivity

First we checked cell growth of *E. coli* CR34, a thymine requiring
mutant, that had been transferred from a minimal medium containing
5 µg/ml thymidine to another containing 0, 0.2 or 0.5 µg/ml thymidine.
We found that although cell mass increased at all concentrations (data
not shown), the number of viable cells decreased considerably after
120 min at thymidine concentrations below 0.5 µg/ml (Fig. 1). After
60 min of transfer, cell cultures at 5, 0.5 and 0.2 µg/ml had doubled,
whereas at 120 min the cultures at 0.5 and 0.2 µg/ml had decreased 30%
and 44% with respect to the one containing 5 µg/ml. When no thymidine
was added to the medium the number of viable cells did not double after

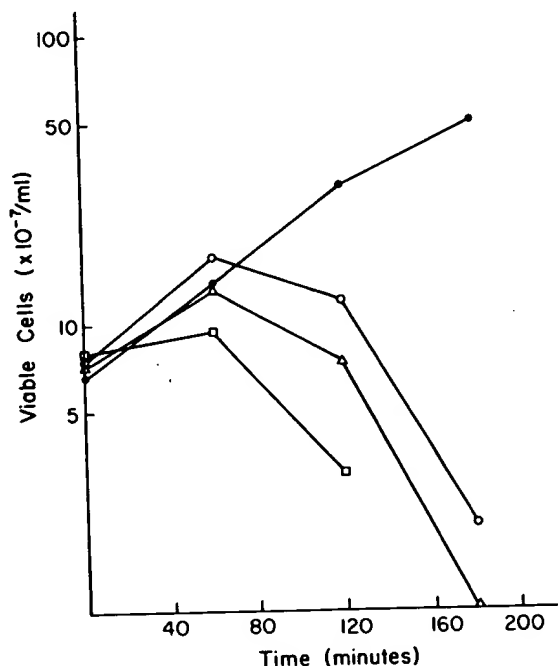


Figure 1. Growth of *E. coli* K-12 CR34 at Different Concentrations of Thymidine

Cells were grown at 37°C in M9-maltose medium supplemented with 1% casamino acids and 5 µg/ml thymidine. At OD₅₉₀ = 0.2, cells were washed and resuspended in fresh medium containing 5 (●), 0.5 (○), 0.2 (△) and 0 (□) µg/ml thymidine. At the times indicated, the number of viable cells was determined.

transfer but were reduced to about 32% after a 120 min incubation. These results suggest that the first doubling of CR34 cells is not appreciably affected if cells are transferred to a medium containing at least 0.2 $\mu\text{g/ml}$ thymidine. With this in mind, we investigated bacteriophage λ infectivity in cells which had been grown at 5 $\mu\text{g/ml}$ thymidine, infected with phage λ and then incubated at various thymidine concentrations. We measured the amount of phage produced under these conditions and found that phage yield is about 43%, 37% and 2% for 0.5, 0.2 and 0 $\mu\text{g/ml}$ thymidine respectively, compared to the culture at 5 $\mu\text{g/ml}$.

(b) λ DNA replicative intermediates

E. coli K-12 CR34 was infected with λ cIIcIII and phage replication allowed for 5-7 min at 37°C in a D_2O -glucose medium (5) containing various concentrations of thymidine. Intracellular λ DNA was isolated from cell lysates and purified through two consecutive CsCl equilibrium centrifugations. In all samples there was a clear shift of the parental phage DNA towards the heavy side of the CsCl gradient, indicating that incorporation of heavy isotope had occurred. Fractions from the heavy side of the parental DNA band were inspected in the electron microscope. A high proportion of the circular molecules were simple replicative intermediates, either double branched (theta), or single branched (sigma) structures (1); a low, but significant (1-6%), proportion of circles contained multiply branched structures in which additional bubbles were present on theta or sigma structures. Properties of these multiply branched molecules have been reported in a recent study (4). Table 1 shows the distribution of the types of circular molecules obtained. First, it is apparent that below 0.5 $\mu\text{g/ml}$ thymidine the percentage of replicative intermediates decreases, however even when no thymidine has been added to the medium, there are still low but significant numbers of replicative intermediates present. This indicates that the amount of thymidine remaining in the cells plus that contained in the yeast extract (see Materials and Methods) is sufficient to allow at least some replication. Second, once the thymidine concentration is reduced below 5 $\mu\text{g/ml}$ the proportion of multiply branched molecules increases. From these findings, and a previous study reporting the occurrence of these structures (4), it is likely that the limited supply of thymidine is crucial in the formation of multiply branched molecules.

(c) Direction of replication of λ DNA replicative intermediates

Fractions containing replicative intermediates were prepared for

TABLE 1

Electron Microscope Analysis of Intracellular λ DNA

Thymidine Added to Medium ($\mu\text{g/ml}$)	Number of Molecules	Percent of Circular Structures			
		Circles*	Replicative Intermediates		
			Theta	Sigma	Multiply Branched Intermediates
5	170	39	35	25	1
0.5	176	35	28	31	6
0.2	172	65	15	15	5
0	173	60	25	10	5

*Includes dimers and monomeric circles containing D-loops. Both of these structures account for about 10 to 21% of all circles.

the electron microscope under conditions of high pH partial denaturation. Partially denatured molecules were photographed at random and the position of branch points determined by alignment of the denaturation patterns with that of mature λ cIIcIII DNA. If we assume that the segments occupied by the known ori mutations give a reliable location for the origin of replication, then, according to recent mapping of these positions (7), the origin is situated 13.75μ from the left end of the 17.06μ λ genome. Assuming that our error in aligning denaturation sites is $\pm 0.25 \mu$ (3), then the region 13.5 - 14.0μ can be used as a gate to classify molecules as either unidirectional or bidirectional. Namely, a molecule with a replicating region spanning the gate was defined as bidirectional. If one branch point fell within the gate, the molecule was scored as unidirectional to the right if the other branch point was to the right of the gate and unidirectional to the left if it was to the left. Molecules with replicated regions that did not span the gate region were rejected. This classification is illustrated in Fig. 2. Here, 38 molecules from an experiment in which no thymidine was added to the medium were photographed at random and positions of branch points in each molecule assigned with the aid of the known denaturation map of mature λ DNA. Replicating segments appear as boxes and the vertical dotted lines crossing all molecules indicate the gate region mentioned above. In this collection, molecules 2-21 are defined as unidirectional to the

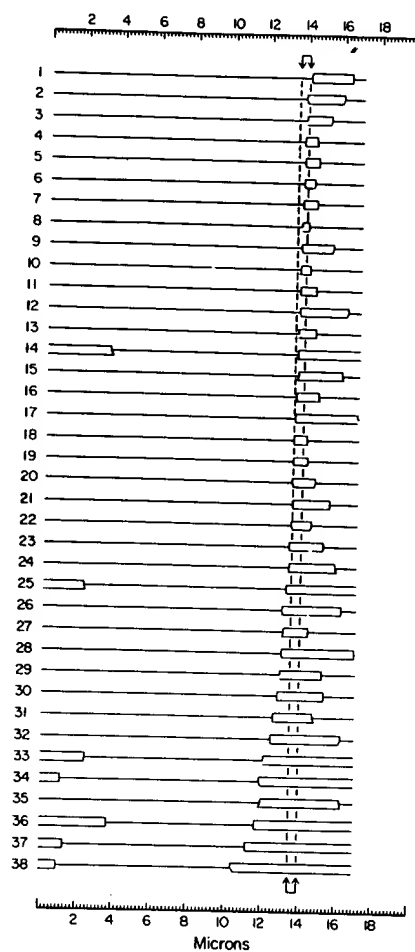


Figure 2. Diagrammatic Representation of the Position of Branch Points in Replicative Intermediates Isolated from Infected Cells With No Added Thymidine Present in the Medium

Circular λ DNA maps were aligned at the mature ends with the aid of the known denaturation pattern of this DNA. They are represented here in a linear fashion for display purposes only. Boxes represent those segments of the λ DNA that have replicated. In the cases where broken boxes are shown, the free end of a tail segment has been positioned on the λ genome using the best alignment of denatured sites present in both the parental DNA and the tail segment. Broken vertical lines indicate the position of the origin region of λ DNA replication ($13.75 \pm 0.25 \mu$). Molecules were sorted according to the position of their left branch points in order to emphasize the predominant unidirectional type of replication in this collection of molecules. Denatured sites and single stranded regions have been omitted for clarity. Molecules have been normalized so that the circular part of each structure is 17.06μ .

right, molecules 22-38 are bidirectional and molecule 1 is a reject, since its replicated region is outside the gate. When this classification is applied to the collection of replicative intermediates from all our experiments, we find that as the thymidine concentration decreases, the proportion of unidirectional types increases from 12% to 52% (Table 2). The bias favoring unidirectional replication to the right also increases as the thymidine concentration is reduced. At 10 $\mu\text{g/ml}$ thymidine this bias is 2:1 whereas below 0.2 $\mu\text{g/ml}$ thymidine it is complete. The bias observed is not due to the activation of a different origin of replication at lower concentrations of thymidine because the number of rejections found in all experiments (Table 2, last column) falls within the range found in earlier studies of five other λ derivatives (3), implying that unidirectional molecules are generated at the normal origin region of λ DNA replication. Moreover double regression plots of bidirectional molecules show that the extrapolated origin sites lie at $13.5 \pm .9$, 14.5 ± 1.0 , $13.9 \pm .5$, and $13.6 \pm .6 \mu$ for 5, 0.5, 0.2, and 0 $\mu\text{g/ml}$ thymidine respectively, and fall within the range of $13.75 \pm 0.25 \mu$, our best estimate of the origin of λ DNA replication. If bidirectional

TABLE 2

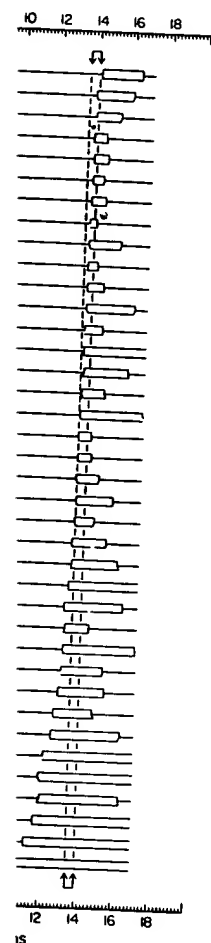
Mode of Replication of λ DNA Replicative Intermediates

Thymidine Added to Medium ($\mu\text{g/ml}$)	Number of Molecules	Mode of Replication (in Percent)			
		Bidirectional	Unidirectional		Rejects
			To Right	To Left	
10 ^a	67	80	8	4	8 ^b
5	44	77	18	5	0
0.5	45	60	38	2	0
0.2	55	53	38	0	9 ^c
0	38	45	52	0	3 ^c

^aData of Schnöls and Irman (1970).

^bOf these 8 rejects, 5 are located to the right of gate, 3 to left.

^cAll of these rejects would be classified as unidirectional to the right if the gate width was enlarged from $\pm 0.25 \mu$ to $\pm 0.50 \mu$ (i.e. twice the estimated error of our alignment procedure).



the Position of Branch Points
olated from Infected Cells
in the Medium

re mature ends with the aid of
. They are represented here in
. Boxes represent those segments
cases where broken boxes are
een positioned on the λ genome
present in both the parental
lines indicate the position of
 $75 \pm 0.25 \mu$). Molecules were
eft branch points in order to
pe of replication in this
nd single stranded regions have
een normalized so that the

replicators also showed a similar bias, then rightward growing points should move further from the origin than their leftward counterparts. To this end we calculated the average positions of left and right branch points (when replication had advanced 59% of the λ genome) by making use of double regression plots of our experimental data. Table 3 shows that the ratio of the average distance of right and left branch points from the origin is appreciably higher only at 0 $\mu\text{g/ml}$ thymidine (2.7) compared to that at 10 $\mu\text{g/ml}$ thymidine (1.3). The large uncertainties attached to the ratios (R/L) shown in the table, reflect the large random deviations from perfectly symmetrical bidirectional growing point movement that has previously been documented (1-3).

DISCUSSION

The first round of bacteriophage λ DNA replication occurs predominantly in a bidirectional fashion. However a fraction of the replicative intermediates are unidirectional and in these replication proceeds either to the right or to the left of the origin (1,2). More recently it has

TABLE 3

Bias in Bidirectionally Replicating λ DNA Molecules

Concentration of Thymidine	Number of Molecules	Average Distance of Branch Points from the Origin at 59% Replication (μ) ^a		
		Right (R)	Left (L)	R/L ^b
10	54	5.62	4.38	1.3 (1.0-1.6)
5	34	6.60	3.40	1.9 (1.2-3.3)
0.5	27	4.97	5.01	1.0 (0.7-1.4)
0.2	29	5.57	4.43	1.3 (1.0-1.5)
0	17	7.29	2.71	2.7 (1.9-4.1)

^aRegression lines were used to estimate, at 95% confidence, the best value for the position of left and right branch points when the separation between them was 10 μ .

^bThe numbers in parentheses represent extreme values (if, at the 95% confidence level, the position of the left branch point was between L_{low} and L_{high} and the right branch point between R_{low} and R_{high} , then the extreme value was calculated as $R_{\text{low}}/L_{\text{high}}$ and $R_{\text{high}}/L_{\text{low}}$).

when rightward growing points their leftward counterparts. It is of interest to compare the positions of left and right branch points of the λ genome) by making use of the present experimental data. Table 3 shows that the separation between the right and left branch points from the origin at 59% replication is 2.7 μ m/ml thymidine (2.7) compared to 1.3 μ m/ml thymidine (1.3) at 59% replication. The large uncertainties attached to these values are due to the large random deviations in the growing point movement that has

A replication occurs predominantly in one direction. The direction of the replicative complex is determined by the direction of the growing point movement (1,2). More recently it has

Table 3. Separation of Branch Points from the Origin at 59% Replication

(R)	Left (L)	R/L ^b
4.38	1.3 (1.0-1.6)	
3.40	1.9 (1.2-3.3)	
5.01	1.0 (0.7-1.4)	
4.43	1.3 (1.0-1.5)	
2.71	2.7 (1.9-4.1)	

^a 95% confidence, the best value for the separation between them

^b The values (if, at the 95% confidence level, the branch point was between R_{low} and R_{high} , then the value is R_{high}/L_{low}).

been established that unidirectionality to the right of the origin is preferred amongst the latter type of replicative intermediates (3). In the present investigation we have shown that this bias is significantly increased at low thymidine concentrations. Under this condition the number of bidirectionally replicating molecules decreases and is replaced by molecules which replicate unidirectionally to the right of the origin. One simple view of initiation of bidirectional DNA replication is to assume the existence of two distinct primer sites on opposite strands of the DNA (at or near the origin region) which will ensure the formation of two replication complexes at each side of the origin and thus lead to a bidirectional mode of DNA replication (8). Unidirectional replication would occur if either the formation of one of the replication complexes was impaired or its progression halted. A bias in growing point propagation in a bidirectional replicator would also result if both complexes were not equivalent. The more efficient complex might start earlier, move more quickly or halt less often. According to this model a more efficient rightward moving replication complex could account for the observations reported above, if this difference in efficiency was exaggerated by a low concentration of thymidine. It is unclear, at present, how thymidine concentration could exert such an effect.

Our results are supported by similar findings made by Edlund *et al.* (9) on *E. coli* DNA replication. These investigators, using the same *E. coli* strain as in our studies, found that changing the thymine concentration from 5 μ g/ml to 0.5 μ g/ml caused a shift in the mode of replication from bidirectional to unidirectional. Furthermore, in a study of plasmid RF100.1, under conditions in which thymine was added after a long thymine starvation period, replicative intermediates showed a unidirectional mode of replication (10) whereas bidirectional replication had been proposed for this plasmid when grown in the absence of such a thymine starvation step (11). It should be stressed that in our system, even when no thymidine is added to the medium, a significant proportion of all replicating intermediates appear to replicate bidirectionally (Table 2). Although there is a large change in the mode of replication such that unidirectional replication to the right is favored, this change is not complete. It is also interesting to note that, at low concentrations of thymidine, multiply branched replicative structures can be isolated (4). It is possible that both the change in directionality and occurrence of multiple branches may be a consequence of the same

factor that affects λ DNA replication when the concentration of available thymidine is reduced. The possibility that SOS induced functions (12) may be playing a role in this process is currently under investigation.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institutes of Health and the American Cancer Society.

REFERENCES

1. Schnös, M. and Inman, R. B. (1970) J. Mol. Biol. 55, 31-38.
2. Valenzuela, M. S., Freifelder, D. and Inman, R. B. (1976) J. Mol. Biol. 102, 569-581.
3. Schnös, M., Denniston, K. J., Blattner, F. R. and Inman, R. B. (1981), submitted for publication.
4. Valenzuela, M. S. and Inman, R. B. (1981), submitted for publication.
5. Ogawa, T., Tomizawa, J. and Fuke, M. (1968) Proc. Natl. Acad. Sci. USA 60, 861-865.
6. Valenzuela, M. S. and Inman, R. B. (1975) Proc. Natl. Acad. Sci. USA 72, 3024-3028.
7. Denniston-Thompson, K., Moore, D. D., Kruger, K. E., Furth, M. E., and Blattner, F. R. (1977) Science 198, 1051-1056.
8. McMacken, R., Ueda, K., and Kornberg, A. (1977) Proc. Natl. Acad. Sci. USA 74, 4190-4194.
9. Edlund, T., Gustafsson, P. and Wolf-Watz, H. (1976) J. Mol. Biol. 108, 295-303.
10. Silver, L., Chandler, M., Boy de la Tour, E. and Caro, L. (1977) J. Bacteriol. 131, 929-942.
11. Perlman, D. and Rownd, R. H. (1976) Nature 259, 281-284.
12. Lark, K. G. and Lark, C. A. (1978) Cold Spring Harbor Symp. Quant. Biol. 43, 537-549.